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APPARENT SPECIFICITY AMONG STREPTOCOCCI AND THE CAUSE OF SOME FAILURES OF MIXED STREPTOCOCCUS VACCINES IN THE TREATMENT OF CHEST COLDS AND ASTHMA*

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IN A PREVIOUS paper[1] it was shown that the varieties of both hemolytic and nonhemolytic streptococci, which are present in the sputum of asthmatic patients, varied more or less from year to year during the period of study, namely, 1918 to 1927. In the present paper, Table I will not only show that this held true for the succeeding period of 1927 to 1930, but also that there was a variation from year to year in the prevalence of the varieties of streptococci that were present in the sputum of patients who had uncomplicated chest colds and who had asthmatic attacks with colds. Often there was a disproportion in the prevalence of the same variety of streptococcus in the same year for the three different conditions. The more and the less prevalent varieties will be noted in this Table.

Another paper[2] showed that vaccines consisting of the streptococci, prevalent during that period, had a curative and possibly specific effect upon colds. There were, however, numerous instances where this mixed vaccine of the more prevalent varieties of streptococci failed to be curative. In the present paper, in Table II, are presented a group of patients in whom one or more doses of a mixed vaccine comprising ten or twelve of the more prevalent varieties of streptococci for that period had no beneficial or curative effect. All of these patients, however, were relieved of their cold or asthma, whichever the case happened to be, following one or two doses of an autogenous sputum vaccine which consisted of one or two of the same varieties of strepto-

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cocci that were present in the mixed vaccine which had failed. The strength or total bacterial count and the dose of both the mixed and autogenous vaccines were the same, but the quantity of each variety of streptococcus in each dose of the autogenous vaccine was five or more times greater than in the mixed vaccine because the former contained only one or two varieties of streptococci whereas the latter contained ten or twelve varieties.

The fact that the mixed vaccine failed to benefit whereas the autogenous vaccine did benefit, would not only be against any nonspecific beneficial effect of vaccine treatment in these cases but would apparently be in favor of specificity among these common varieties of streptococci. Furthermore, it is evident that larger quantities of the proper bacteria in a vaccine may be beneficial when smaller quantities fail. This is one reason why the mixed vaccine, even though it contained the proper or causative bacteria along with other varieties, sometimes failed; the quantity of the causative streptococcus was too small to be of benefit.

In another paper[3] it was stated that, following the administration of a vaccine consisting of the more prevalent streptococci, 59 per cent of those patients who were very susceptible to colds and to asthma associated with colds obtained freedom or comparative freedom from colds and asthma. In another 36 per cent the frequency of colds and asthmatic colds was reduced by 50 or 75 per cent, and in the remaining 5 per cent there was no benefit. Since this vaccine consisted of those streptococci which represented between 85 and 90 per cent of the prevailing varieties, it was assumed that the less prevalent varieties or the remaining 10 or 15 per cent of those streptococci which were encountered might be the cause of some of the chest colds and asthmatic colds in those patients who did not obtain freedom following the vaccine consisting of the more prevalent streptococci.

In the present paper, Table III will prove that this assumption was correct in the group of cases therein presented. After these patients had completed a course of vaccine, which consisted of the ten or twelve more prevalent varieties of streptococci and which was given as a preventive against chest colds and asthmatic colds, they had either a chest cold or an attack of asthma with a cold or their prevailing asthma was not benefited, according as the case happened to be. The sputum obtained at this time obtained one or more of the less prevalent varieties of streptococci or those in the 10 or 15 per cent group which were not included in the preventive vaccine; none of the more prevalent streptococci or those in the 85 or 90 per cent group which made up the preventive vaccine were recovered from these sputa. Furthermore, prompt benefit or relief from the cold or asthma followed one dose of the autogenous vaccine which consisted of these less prevalent streptococci. These instances, especially the asthmatics, would seem to illustrate specificity among these common streptococci and in those who were susceptible to colds to explain the failure of the preventive vaccine.

Table IV in this paper is identical with Table III with the one exception, namely, that the sputum of these cases did contain, along with less prevalent varieties which were not present in the preventive vaccine, one variety of the more prevalent streptococci which was present in the preventive vaccine.

These cases, therefore, would seem to illustrate specificity as did the cases in Table III and the reason for failure of the preventive vaccine might be due either to insufficient treatment with the one causative variety that was present in the mixed vaccine or to the varieties of streptococci found in the sputum that were not present in the vaccine or to a combination of both.

It has already been stated, in Table II, and suggested, in Table IV, that one cause of failure of a vaccine, which consists of several varieties of organisms even though the causative variety is included, is that in the composite vaccine the causative organism is present in too small a quantity to be of value. In other words, although the dose of the composite vaccine is as large as can be given, the dose of each of the varieties that make up the total is proportionally small and may be too small to immunize successfully. The cases presented in Table V not only seem to illustrate this point but also to illustrate the advisability of using an autogenous vaccine when a mixed vaccine fails. Furthermore, these instances are against any nonspecific effect of vaccine treatment, and they would seem to favor specificity among the common varieties of streptococci.

The bacteriologic technic employed in this paper has been fully described in a previous paper,[1] therefore only an outline is necessary at this time. Washed masses of thick sputum, which were raised in the early morning and collected in sterile bottles, were shaken in dextrose bouillon and then inoculated into human blood agar, which was poured into Petri dishes. After the blood agar plates were incubated from twenty to thirty-six hours, depending upon the rapidity of growth, they were examined for types of colonies. Numerous colonies were picked and macerated in dextrose bouillon and incubated for twenty-four hours, after which it was examined. Cultures that were typical streptococci in pure culture were inoculated into litmus serum waters. Therefore, examination of the human blood agar plate revealed the presence or absence of pigment and hemolysis for the various colonies; examination of the dextrose bouillon culture determined the morphology and staining characteristics of the organism; and the litmus serum waters in the case of streptococci differentiate both the hemolytic and the nonhemolytic strains into eight subgroups or varieties. No attempt was made to identify bacilli except for morphology, pigment, motility, and staining characteristics.

The bacterial count of all the vaccines was fifteen hundred million organisms per cubic centimeter or one hundred million per minim. All vaccines were given at weekly intervals; the first dose was three minims and each week the dose was increased one minim. In the mixed vaccine composed of the more prevalent streptococci, there were many strains of each variety because all strains isolated from each and every patient were pooled, thereby negating to a great extent any chance that an autogenous streptococcus might be more effective in a given case than the same strain recovered from another patient.

All of the patients represented one of three pathologic conditions, namely, those who had uncomplicated chest colds, others who had asthmatic attacks only when they had a chest cold with free intervals between their colds and asthmatic attacks, and patients who had continuous or chronic asthmatic

bronchitis. These three conditions appear in each table throughout the paper. All of the cases had been subject to these conditions for several years, therefore the sequence of symptoms was well established, and all have been followed up since so that the result of treatment is unquestionable.

These studies were made during the successive cold seasons, fall, winter, and spring of each year, so that each period extends over a part of two successive calendar years, namely, September to April inclusive of 1927 and 1928, 1928 and 1929, and 1929 and 1930. In Table I, a total of 100 patients was studied during each period. The figures in the columns headed by the various names of the streptococci refer to the number of patients from whom each variety of streptococcus was recovered rather than to the number of strains of that variety. For instance, in the column headed subacidus, the figure 11 means that the variety subacidus was recovered from the sputum of eleven patients, and not that eleven strains of subacidus were recovered. Naturally, several strains or colonies of the same variety would be picked from a plated culture, but the number of strains does not enter into this paper. Other than this, the table is self-evident and needs no further explanation.

It is noted that the varieties of hemolytic streptococci, namely subacidus and anginosus, and of the nonhemolytic varieties, namely ignavus, were the only ones that were consistently most prevalent in all three periods. The variety of hemolytic streptococcus, namely pyogenes, and of the nonhemolytic streptococci, namely salivarius, fecalis and nonhemolytic Type I, were next in consistent frequency in all periods although with these there was considerable variation in frequency for the three different periods. At any rate all of these seven varieties of streptococci were sufficiently prevalent on the percentage basis to be included in the mixed vaccine of more prevalent varieties for all three periods.

The varieties of hemolytic streptococci, namely infrequens and hemolytic Type I, were sufficiently prevalent during the periods 1927 and 1928 and 1928 and 1929 to be classed among the more prevalent streptococci for these two periods, but in the period 1929 and 1930 these organisms were recovered only about one-half as frequently. Because of their frequency during the first two periods, they were included in the mixed vaccine of more prevalent streptococci for all three periods. The nonhemolytic variety mitis was among the most prevalent streptococci in the period 1928 and 1929; although it was only one-half as prevalent in 1929 and 1930, on the percentage basis, it was still among the more prevalent streptococci but in the first period it was among the less frequent varieties. Since in the previous study[1] this variety, namely mitis, had been very prevalent for several years it was included in the mixed vaccine during the period of this study.

Therefore, the ten varieties of streptococci which were discussed in the two previous paragraphs were incorporated into the mixed vaccine of more prevalent streptococci that was employed throughout the present study both as a preventive and as a curative, and in the accompanying tables it will be referred to as a "mixed vaccine."

Although the varieties hemolytic equi and nonhemolytic equinus were not frequent in the period 1927 and 1928, they were among the more frequent

TABLE I

VARIATION AND PREVALENCE OF STREPTOCOCCI IN THE SPUTUM OF CHEST COLDS AND ASTHMA

YEAR	DIAGNOSIS	PATIENTS	HEMOLYTIC STREPTOCOCCI						NONHEMOLYTIC STREPTOCOCCI							
			SUBACIDUS	ANGINOSUS	PYOGENES	INFREQUENS	EQUI	HEMOLYTIC TYPE I	HEMOLYTIC TYPE II	HEMOLYTIC TYPE III	IGNAVUS	SALIVARIUS	FECALIS	MITIS	EQUINUS	NONHEMOLYTIC TYPE I	NONHEMOLYTIC TYPE II	NONHEMOLYTIC TYPE III
1927-28	Chest Colds	35	11	10	4	6	21	15	0	3	18	12	7	21	21	9	1	0
	Asthma with Colds	25	10	21	1	15	1	3	0	0	13	4	9	21	0	12	12	1
	Chronic Asthma	40	18	11	6	4	3	1	4	21	15	14	11	3	6	11	1	0
1928-29	Chest Colds	40	15	13	7	13	8	8	6	3	17	13	8	11	8	15	21	6
	Asthma with Colds	25	12	6	4	4	1	21	4	21	15	1	4	21	3	13	3	1
	Chronic Asthma	35	14	8	8	6	10	6	1	1	9	8	11	10	8	8	1	0
1929-30	Chest Colds	35	17	14	4	7	3	4	0	21	21	6	15	6	3	4	0	1
	Asthma with Colds	25	9	21	4	1	1	1	0	0	12	3	13	21	21	3	1	0
	Chronic Asthma	40	28	10	6	0	10	21	0	1	25	6	6	4	3	15	0	3

varieties during 1928 and 1929, therefore, these two varieties of streptococci were added to the mixed vaccine for the periods 1928 and 1929 and 1929 and 1930. It is noted that in 1929 and 1930 the variety equi was still very prevalent but the variety equinus was among the less prevalent varieties.

The four varieties hemolytic and nonhemolytic Types II and III, respectively, may be discussed together since they all were consistently less prevalent and yet they all showed considerable variation in frequency for the different periods of study. For instance hemolytic Type II was not recovered at all in the last period but it was encountered in eleven patients during the second period and in only four during the first period.

Variation in the frequency of the same variety of streptococcus in the same period is still more marked for the three different pathologic conditions, namely, uncomplicated chest colds, asthma with chest colds and chronic asthma or asthmatic bronchitis. The greatest disproportion was noted with Streptococcus anginosus in the first and third periods of study, when it was recovered from the sputum of only two cases of asthma with colds, whereas it was recovered from ten or more patients with uncomplicated colds and with asthmatic bronchitis. Streptococcus infrequens in the third period was recovered from seven patients with chest colds but from only one patient with asthmatic colds and from none of the chronic asthmatics. Streptococcus hemolyticus Type II during the first period was recovered from chronic asthmatics only, whereas in the second period it was recovered only once from chronic asthmatics but from four to six cases of the other two conditions. Streptococci equinus and nonhemolyticus Type II in the first period were recovered in not more than two cases of asthmatic colds, but in the other two conditions these two organisms were recovered six or more times.

There was a marked disproportion in the frequency of subacidus, equi and ignavus in the third period; subacidus was much more prevalent in the asthmatic cases and less prevalent in the asthmatic colds, equi was less prevalent in the chest colds than in the other two conditions, and ignavus was nearly twice as prevalent in the cases having chronic asthma and uncomplicated chest colds as in those having asthma with colds. During the same period certain varieties were encountered more often in one condition than in the other two conditions whereas in other periods they were encountered less often. In fact, if the three conditions were considered separately, a vaccine composed of the more prevalent varieties of streptococci would of necessity be different for each condition for the same and for different periods.

From the sputum of those patients who had chest colds and chronic asthma the total number of all varieties of both hemolytic and nonhemolytic streptococci that were recovered was very nearly the same for all three periods of study. However, in the patients who had asthma with chest colds the total number of varieties of both hemolytic and nonhemolytic streptococci was much less in proportion for all three periods. In fact, only about one-half as many of the varieties of streptococci were recovered in those patients who had asthma associated with colds as in the other two conditions.

In Table II are presented twenty-seven patients who were treated one or more times with the mixed vaccine for its curative effect but without any

benefit. At the same visit or within the next day or two a specimen of their sputum was obtained and from it an autogenous vaccine was made and the varieties of streptococci were identified. The following week the patients were given a dose of their autogenous vaccine. The bacterial count and the dose of both the mixed vaccine which did not benefit and of the autogenous vaccine which did benefit was the same. The only apparent difference in the two kinds of vaccine was that the dose of mixed vaccine contained eight or ten varieties of streptococci that were not present in the sputum at that time in addition to the one or two varieties that were in the sputum. Thus each dose of the mixed vaccine consisted of a small number of each of ten or twelve varieties of streptococci whereas the same dose of the autogenous vaccine consisted of a much larger number of one or two of these same varieties.

TABLE II

TREATMENT WITH MIXED VACCINE VERSUS AUTOGENOUS VACCINE

PATIENT	DIAGNOSIS	TREATMENTS WITH MIXED STREPTOCOCCUS VACCINE	RESULT	TREATMENT WITH AUTOGENOUS STREPTOCOCCUS VACCINE	RESULT, FIRST TREATMENT	RESULT, SECOND TREATMENT
1	Chest Cold	1	No benefit	Fecalis	Relieved	
2	Chest Cold	1	No benefit	Infrequens	Relieved	
3	Chest Cold	1	No benefit	Ignavus	Relieved	
4	Chest Cold	1	No benefit	Fecalis, Equi	Relieved	
5	Chest Cold	1	No benefit	Salivarius	Relieved	
6	Chest Cold	1	No benefit	Ignavus, Anginosus	Relieved	
7	Chest Cold	1	No benefit	Subacidus, Ignavus	Relieved	
8	Asthma with a Cold	1	No benefit	Infrequens	Relieved	
9	Asthma with a Cold	1	No benefit	Anginosus, Nonhemolyticus I	Relieved	
10	Asthma with a Cold	2	No benefit	Subacidus, Salivarius	Relieved	
11	Asthma with a Cold	2	No benefit	Subacidus, Infrequens	Relieved	
12	Asthma with a Cold	2	No benefit	Salivarus	Improved	Relieved
13	Asthma with a Cold	1	No benefit	Hemolyticus I, Nonhemolyticus I	Improved	Relieved
14	Asthma with a Cold	2	No benefit	Ignavus, Hemolyticus I	Improved	Relieved
15	Asthma with a Cold	2	No benefit	Ignavus	Improved	Relieved
16	Asthma with a Cold	2	No benefit	Ignavus	Improved	Relieved
17	Chronic Asthma	2	No benefit	Ignavus	Relieved	
18	Chronic Asthma	1	No benefit	Mitis	Relieved	
19	Chronic Asthma	1	No benefit	Fecalis	Improved	Relieved
20	Chronic Asthma	3	No benefit	Anginosus, Ignavus	Improved	Relieved
21	Chronic Asthma	1	No benefit	Fecalis	Improved	Relieved
22	Chronic Asthma	2	No benefit	Ignavus	Improved	Relieved
23	Chronic Asthma	1	No benefit	Fecalis	Improved	Relieved
24	Chronic Asthma	1	No benefit	Anginosus, Salivarius	Improved	Relieved
25	Chronic Asthma	1	No benefit	Infrequens, Fecalis	Improved	Relieved
26	Chronic Asthma	2	No benefit	Ignavus, Nonhemolyticus I	Improved	Relieved
27	Chronic Asthma	2	No benefit	Infrequens, Equinus	Improved	Relieved

The first seven patients who had uncomplicated chest colds were not benefited by one dose of the mixed vaccine. A week later, a dose of their autogenous sputum vaccine was given and within the following week these patients were so much improved of their chest colds that they deemed further treatment unnecessary. In four of these patients only one variety of strep-

tococcus, that was present in the mixed vaccine, was recovered from the sputum so that in the autogenous vaccine the quantity of this causative streptococcus was at least ten times greater than in the mixed vaccine. In the other two patients two varieties, that were in the mixed vaccine, were recovered from the sputums, so that in these cases the number of the causative streptococci was at least five times greater for each variety in the autogenous than in the mixed vaccine.

The next nine patients who had asthma only with chest colds were not benefited by one and some not by two doses of the mixed vaccine, whereas four were so much relieved by one dose of their autogenous sputum vaccine that they did not require a second dose and the other five were much benefited by one dose and relieved by a second dose of their autogenous vaccine. In four of these cases only one variety of streptococcus was isolated from the sputum and in the other five cases only two varieties were isolated, so that in the former cases the autogenous vaccine contained the causative streptococcus in ten times the quantity that it was present in the mixed vaccine and in the latter cases in at least five times the quantity.

The remaining eleven patients had chronic asthma; six were given one dose of the mixed vaccine without benefit, and four who were given two doses and one who was given three doses were not benefited. Two of these cases, however, were so much benefited by one dose of an autogenous vaccine that they did not report for a second treatment and the others were benefited by one dose and so much relieved by a second dose that they reported for no more treatment. In six of these patients only one variety of streptococcus was recovered from the sputum and in the other five patients only two varieties were recovered. Therefore, as in the preceding cases the count of the causative organism in the autogenous vaccine was five to ten times greater than in the mixed vaccine.

In these cases it is noted that none of the less prevalent varieties of streptococci were isolated from the sputum, but that eleven out of the twelve more prevalent varieties that comprised the mixed vaccine were recovered. Since the only apparent difference between the two types of vaccine was the quantity of the various organisms in each, it is evident that the mixed vaccine failed because it was too weak as regards each respective causative variety of streptococcus in that particular case. In other words, for these patients the particular causative variety of streptococcus was not present in sufficient amounts in the mixed vaccine for one or two doses to raise immunity.

Since the bacterial count of both kinds of vaccine was the same and the dose was the same, benefit from one and not from the other would surely be evidence against any nonspecific effect of vaccine treatment and would tend to support specificity among these streptococci.

In Table III is presented a group of patients who had completed a course of treatment with the mixed vaccine which consisted of the more prevalent varieties of streptococci at that time and which, as already stated, included the varieties met with in 85 to 90 per cent of such patients. With the patients who had uncomplicated chest colds and asthma only with chest colds, this vaccine was given as a preventive and with the chronic asthmatics it was

given as a curative. During the succeeding few weeks, however, the first two kinds of patients had chest colds and the asthmatics continued to have asthma. From the sputum of all of these patients were recovered only those varieties of streptococci that were among the less prevalent ones or the ones that were met with in 10 to 15 per cent of the cases and consequently were omitted from the mixed vaccine.

All of those patients who had a chest cold, with the exception of the first one who was not treated, were relieved of their cold inside of one week following a dose of their autogenous vaccine which consisted of one of the less prevalent streptococci, as noted in Table III. Three of those patients who had asthma with a chest cold were relieved within a week and the other patient was improved following one dose of an autogenous vaccine, and was relieved following a second dose. From the sputum of the three patients who were

TABLE III

CHEST COLDS AND ASTHMA CAUSED BY THE LESS PREVALENT STREPTOCOCCI AFTER PREVENTIVE TREATMENT WITH THE MORE PREVALENT STREPTOCOCCI

PATIENT	DIAGNOSIS	STREPTOCOCCI IN SPUTUM	TREATMENT WITH AUTOGENOUS VACCINE	
			FIRST DOSE	SECOND DOSE
1	Chest Cold	Equi, Equinus	No treatment	
2	Chest Cold	Nonhemolytic II	Relief	
3	Chest Cold	Hemolytic II	Relief	
4	Chest Cold	Nonhemolytic II	Relief	
5	Chest Cold	Hemolytic III	Relief	
6	Chest Cold	Hemolytic III	Relief	
7	Chest Cold	Equi	Relief	
8	Asthma with a Cold	Nonhemolytic III	Relief	
9	Asthma with a Cold	Nonhemolytic III	Relief	
10	Asthma with a Cold	Hemolytic II, Nonhemolytic II	Improved	Relief
11	Asthma with a Cold	Equinus	Relief	
12	Chronic Asthma	Equi, Equinus, Nonhemolytic II	Improved	Relief
13	Chronic Asthma	Equinus	Improved	Relief
14	Chronic Asthma	Equi	Improved	Relief
15	Chronic Asthma	Hemolytic II, Nonhemolytic II	Improved	Relief
16	Chronic Asthma	Hemolytic II, Nonhemolytic II	Improved	Relief

relieved following one dose of their autogenous vaccine only one of the less prevalent streptococci was recovered and from the other patient two varieties were recovered. These cases illustrate why the mixed vaccine of more prevalent streptococci failed to prevent the current cold. It is of sufficient interest to warrant mentioning the fact that none of these patients had another chest cold or asthmatic cold during the following six months or longer, whereas, since they had been accustomed to have four or more colds each year, these patients would have expected to have two colds during this interval of freedom.

The last five patients in Table III all had chronic asthma which was improved following one dose of an autogenous vaccine and was relieved following a second dose. In two of these cases only one of the less prevalent streptococci was recovered from the sputum, in two cases two varieties, and in the remaining case three varieties were recovered. These cases of asthma, which had not been benefited by several doses of the mixed vaccine, but did improve

rapidly following one dose and were relieved after a second dose of the specific organism in their sputum, are very much against any nonspecific beneficial effect of vaccine treatment and present evidence strongly in favor of specificity.

The patients in Table IV, as was the case in Table III, were given a course of mixed vaccine consisting of the more prevalent streptococci as a preventive against chest colds and as a curative in chronic asthma. These patients, as did those presented in Table III, who were subject to chest colds, did shortly after the treatment have a chest cold, and those who were having chronic asthma were not benefited. From the sputum of these patients were recovered one variety of the more prevalent streptococci which was present in the mixed vaccine and one or more of the less prevalent varieties like those in Table III. In all instances the one variety of the more prevalent streptococci is mentioned first in each case. For instance, from the sputum of Patient 1, fecalis is one of the more prevalent and equi one of the less prevalent varieties for that period, and in No. 2 subacidus was the more prevalent, whereas hemolytic III and nonhemolytic II were less prevalent.

TABLE IV

CHEST COLDS AND ASTHMA CAUSED BY STREPTOCOCCI AFTER A COURSE OF MIXED VACCINE TREATMENT

PATIENT	DIAGNOSIS	VARIETIES OF STREPTOCOCCI IN SPUTUM	TREATMENT WITH AUTOGENOUS VACCINE	
			FIRST DOSE	SECOND DOSE
1	Chest Cold	Fecalis, Equi	Relieved	
2	Chest Cold	Subacidus, Hemolytic III, Nonhemolytic II	Relieved	
3	Chest Cold	Anginosus, Equi, Equinus	Relieved	
4	Chest Cold	Ignavus, Equinus	Relieved	
5	Chest Cold	Ignavus, Equinus	Relieved	
6	Chest Cold	Mitis, Nonhemolytic II	No Treatment	
7	Asthma with a Cold	Ignavus, Hemolytic III	Improved	Relieved
8	Asthma with a Cold	Ignavus, Nonhemolytic III	Improved	Relieved
9	Asthma with a Cold	Ignavus, Equi	Relieved	
10	Asthma with a Cold	Ignavus, Equinus	Relieved	
11	Asthma with a Cold	Ignavus, Equinus	Improved	Relieved
12	Asthma with a Cold	Ignavus, Nonhemolytic III	Relieved	
13	Asthma with a Cold	Fecalis, Hemolytic II, Hemolytic III	Relieved	
14	Asthma with a Cold	Infrequens, Equi, Hemolytic II, Equinus	Relieved	
15	Asthma with a Cold	Fecalis, Nonhemolytic II	No Treatment	
16	Chronic Asthma	Fecalis, Hemolytic II, Equinus	Improved	Relieved
17	Chronic Asthma	Subacidus, Equi, Equinus, Nonhemolytic II	Improved	Relieved
18	Chronic Asthma	Fecalis, Nonhemolytic II, Nonhemolytic III	Improved	Relieved
19	Chronic Asthma	Salivarius, Equi, Equinus	Improved	Improved
20	Chronic Asthma	Ignavus, Nonhemolytic II	Improved	Improved
21	Chronic Asthma	Ignavus, Nonhemolytic III	Improved	Improved

It is noted, as was the case in Table III, that all of the patients who were treated with their autogenous vaccine were either relieved of their condition or improved during the week following their first dose of vaccine and those who were only improved were relieved following a second dose. For reasons already stated the asthmatic cases would present strong evidence against any

nonspecific vaccine effect and on the contrary they would support specificity among these streptococci.

A point that arises and needs discussion in Table IV is whether in each patient's sputum the one of the more prevalent varieties or the one or more of the less prevalent varieties of streptococci were encountered in greater numbers. The number of colonies of each variety of streptococcus that was picked from the plated culture will answer this question and also will help to determine whether the current colds and continuous asthma was more likely due to insufficient treatment with the particular variety of streptococcus that was more prevalent and therefore was present in the mixed vaccine or more likely due to the streptococci that were less prevalent and consequently not present in the mixed vaccine.

In the sputum of Patient No. 1 there were three colonies of Streptococcus fecalis to one colony of equi and in Patient No. 11 there were four colonies of Streptococcus ignavus to one colony of equinus. Therefore, in these two patients it is fair to assume that the two varieties of streptococci that so outnumbered the other two less frequent varieties probably played a greater part in the cause of the symptoms and consequently the mixed vaccine did not prevent chest colds because these patients required a larger quantity of the two more prevalent streptococci than was present in the mixed vaccine.

From the sputum of Patient No. 2 three times as many colonies of the less prevalent streptococci were isolated as of the more prevalent and from Patient No. 13 four times as many of the former as of the latter, and from Patient No. 14 six colonies of the less prevalent were isolated to one of the more prevalent. Therefore in these cases it is probably fair to assume that the less prevalent streptococci played a greater part in the causation of symptoms and, if this is so, they would support specificity.

In four other cases twice as many colonies of the less prevalent streptococci were isolated from the sputum as of the more prevalent and in the remaining twelve cases the colonies of both the more and the less prevalent were about equally divided. Therefore, in these cases it is impossible to state whether the less prevalent or the more prevalent streptococci played a greater part in the cause of symptoms, and it is more fair to assume that both were responsible.

In Table V are presented a group of patients who, after they had been given a course of the mixed vaccine as a preventive against chest colds or as a curative for chronic asthma, as the case happened to be, either had a chest cold or continued to have asthma, and from the sputum only the more prevalent streptococci which were present in the mixed vaccine were isolated.

Since the nine patients who had chest colds were all relieved inside of a week by one dose of their autogenous sputum vaccine, which consisted of from one to three varieties of the streptococci that comprised the mixed vaccine, it is apparent that these organisms caused the colds. In those cases where the sputum grew only one variety of streptococcus, that particular organism was present in the autogenous vaccine in at least ten times the quantity that was present in the mixed vaccine. Similarly where the sputum grew only two varieties of streptococci, each was present in the autogenous

vaccine in five times the quantities in the mixed vaccine. These same statements hold true for the eight patients who had a subsequent chest cold complicated by asthma.

In these cases it would seem evident that the particular causative streptococcus was not present in sufficient quantity in the mixed vaccine to produce an immunity. As a matter of fact, the final treatment in a course of the preventive mixed vaccine did not contain as large a dose of each component variety of streptococcus as did one treatment of the autogenous vaccine. This fact would not only be against any nonspecific effect of vaccine treat-

TABLE V

CHEST COLDS AND ASTHMA CAUSED BY THE MORE PREVALENT STREPTOCOCCI AFTER TREATMENT WITH THESE SAME ORGANISMS

PATIENT	DIAGNOSIS	VARIETIES OF STREPTOCOCCI IN AUTOGENOUS VACCINE	RESULT OF TREATMENT		
			FIRST DOSE	SECOND DOSE	THIRD DOSE
1	Chest Cold	Subacidus, Ignavus	Relieved		
2	Chest Cold	Subacidus, Pyogenes	Relieved		
3	Chest Cold	Subacidus, Ignavus	Relieved		
4	Chest Cold	Fecalis, Salivarius	Relieved		
5	Chest Cold	Subacidus, Ignavus	Relieved		
6	Chest Cold	Anginosus, Salivarius	Relieved		
7	Chest Cold	Salivarius	Relieved		
8	Chest Cold	Subacidus, Anginosus, Ignavus	Relieved		
9	Chest Cold	Ignavus	Relieved		
10	Asthma with a Cold	Subacidus	Relieved		
11	Asthma with a Cold	Nonhemolytic I	Relieved		
12	Asthma with a Cold	Anginosus, Nonhemolytic I	Relieved		
13	Asthma with a Cold	Equi, Ignavus, Salivarius	Improved	Relieved	
14	Asthma with a Cold	Pyogenes, Ignavus	Improved	Relieved	
15	Asthma with a Cold	Subacidus, Pyogenes	Improved	Relieved	
16	Asthma with a Cold	Pyogenes	Improved	Relieved	
17	Asthma with a Cold	Infrequens, Ignavus, Fecalis	Improved	Relieved	
18	Chronic Asthma	Hemolytic I, Nonhemolytic I	Improved	Relieved	
19	Chronic Asthma	Subacidus, Ignavus	Improved	Relieved	
20	Chronic Asthma	Subacidus, Ignavus	Improved	Improved	Relieved
21	Chronic Asthma	Subacidus, Equi, Hemolyticus I, Equinus	Improved	Improved	Relieved
22	Chronic Asthma	Subacidus, Anginosus, Equi	Improved	Relieved	
23	Chronic Asthma	Nonhemolytic I	Improved	Relieved	
24	Chronic Asthma	Hemolytic I, Ignavus, Nonhemolytic I	Improved	Improved	Relieved
25	Chronic Asthma	Hemolytic I, Nonhemolytic I	Relieved		

ment in these cases but it would favor specificity, and also it would explain why the mixed vaccine sometimes failed to prevent chest colds and asthma associated with colds.

From the sputum of the eight patients with chronic asthma, after completion of a course of treatment with the mixed vaccine without benefit, were isolated from one to three of the varieties of streptococci that comprised the mixed vaccine. An autogenous vaccine of these from one to three varieties as the case happened to be was followed by improvement; one patient was relieved after one dose, four were relieved after a second dose, and three were relieved after a third dose. The statements in the two preceding paragraphs apply equally as well to this group of cases; specificity is more evident since repeated small doses of the causative organism which was present in large

doses of the mixed vaccine did not benefit, whereas one large dose did, and this would likewise explain why the mixed vaccine was not curative in some cases.

SUMMARY AND CONCLUSIONS

Not only is there a variation from year to year among the varieties of streptococci that are present in the sputum of asthmatic patients[1] but also in the sputum of patients who have uncomplicated chest colds and chest colds complicated by asthma. There is more or less variation in the prevalence of the same variety of streptococcus in the same year among the three different conditions and in a previous paper[4] was shown a similar variation in chest colds, even in the same patient. Nevertheless, some varieties of streptococci were more prevalent than others for a certain period.

A mixed vaccine comprising the more prevalent varieties of streptococci for a given period, as shown in previous papers, has considerable value as a curative[1] and still more value as a preventive[3] in chest colds and asthma. The present paper shows that in some patients in whom this vaccine failed the failure was due to the presence of an insufficient quantity of the particular causative streptococcus in the mixed vaccine or, in other words, relief followed the larger amounts of the causative streptococcus that were present in the autogenous vaccine than were present in the mixed vaccine. In other patients failuse of the mixed vaccine was due to the fact that the present illness was caused by one or more of the less prevalent streptococci which were not present in the mixed vaccine.

The results from treatment in all of the 89 patients presented in this paper are against any nonspecific effect of vaccines and to the contrary support specificity. In many instances vaccine treatment was undoubtedly specific and showed a specificity among the common streptococci.

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ACTIVE IMMUNIZATION AGAINST TYPHOID FEVER, WITH PARTICULAR REFERENCE TO AN INTRADERMAL METHOD*

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VERY little doubt exists today concerning the value of active immunization against typhoid fever by means of the administration of typhoid vaccine. Any difference of opinion which exists concerns itself either with the method of administration or with the type of vaccine used. The method most frequently employed[1] consists usually of the subcutaneous administration at 5 to 7 day intervals of either three or four doses of a saline suspension containing typhoid bacilli, paratyphoid A and B, in a concentration of 1000 million typhoid and 500 million each of paratyphoid A and B per cubic centimeter and given in doses of ½ c.c. as the first dose, and 1 c.c. as the subsequent doses. These injections are often followed by local and general symptoms which, though never alarming, are nevertheless uncomfortable and annoying. During the past year, I have been employing a method which seemed to offer sufficient advantages over methods previously used to warrant its report.

The experimental studies upon which the use of this method was based, constituted part of a program of investigation concerning the relationship of the skin to processes of immunity, especially to antibody production.[2] Clinical investigations and experimental data accumulating within the past decade have all tended to support the idea that the skin plays an important rôle immunologically. In order to investigate this subject further, in conjunction with Elizabeth M. Yagle, Ph.D., and Stuart H. Rogers, A.B., of the staffs of the Graduate Hospital and Research Institute of Cutaneous Medicine, I studied comparatively the antibody response which followed different methods of administration of a triple typhoid vaccine to groups of young normal healthy females ranging in ages between eighteen and twenty-five. The detailed results of this investigation will appear in another publication,[3] but certain of these results pertinent to the present paper will be briefly summarized below. The first group of 12 individuals were subdivided into 3 subgroups of 4 each; 2 of these subgroups received the vaccine by the intramuscular and subcutaneous routes, the doses being 0.5, 1.0, 1.0, and 1.0 c.c. at weekly intervals, whereas the third subgroup received the same vaccine intradermally according to the following schedule of doses: 0.05, 0.1, 0.15,

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and 0.2 c.c. at weekly intervals. Blood samples were taken before and at frequent intervals up to twelve months after the injection, and studied for agglutinins and complement fixing antibodies. Results of these studies showed that although the total dosage by the intradermal method was $\frac{1}{7}$ by that of other methods of administration, nevertheless, the antibody response was equally as good. This is exemplified in the upper four rows in Table I. Similar studies carried out three and four months later upon two other groups of 15 and 29 individuals, respectively, showed essentially the same comparative results with the exception that the antibody response was generally weaker, as

TABLE I

COMPARATIVE SERUM AGGLUTININ TITERS AFTER VARIOUS METHODS OF ADMINISTRATION OF MIXED TYPHOID VACCINE, THREE MONTHS AFTER INITIAL INJECTION

Dose of intradermal injections: 50, 100, 150, and 200 millions at weekly intervals. Dose of subcutaneous and intramuscular injections: 500, 1,000, and 1,000 and 1,000 millions at weekly intervals.

TYPHOID			PARA A			PARA B		
INTRA-DERMAL	SUBCU-TANEOUS	INTRA-MUSCULAR	INTRA-DERMAL	SUBCU-TANEOUS	INTRA-MUSCULAR	INTRA-DERMAL	SUBCU-TANEOUS	INTRA-MUSCULAR
160	20	80	320	80	40	80	80	160
80	320	80	160	320	40	160	320	40
80	80	20	80	80	640	40	20	40
40	320	160	40	80	80	40	160	10
40	40	20	80	80	80	80	40	160
40	20	20	40	20	80	80	80	80
80	20	10	40	40	80	80	80	80
	20	20		40	80		40	80

TABLE II

COMPARATIVE SERUM AGGLUTININ TITERS AFTER VARIOUS METHODS OF ADMINISTRATION OF A MORE POTENT TYPHOID VACCINE, THREE MONTHS AFTER INITIAL INJECTION

Dosage: 50 million, 100 million, 150 million and 200 million at weekly intervals.

TYPHOID			PARA A			PARA B		
INTRA-DERMAL	SUBCU-TANEOUS	INTRA-MUSCULAR	INTRA-DERMAL	SUBCU-TANEOUS	INTRA-MUSCULAR	INTRA-DERMAL	SUBCU-TANEOUS	INTRA-MUSCULAR
160	1280	640	320	320	80	80	160	80
640	1280	2560	80	40	80	160	80	80
1280	640	5120	40	40	80	40	20	160
640	320	1280	320	40	80	320	40	80
1280	5120		80	40		160	80	
1280	1280		80	80		160	80	
5120			40			160		
80			40			160		
320			160			80		

is exemplified in the lower rows of Table I. Since we were using the same vaccine throughout, it seemed evident that the difference in response was due to a difference in the age of the vaccine. This view was substantiated by the use of a vaccine freshly prepared from the same strain of organisms and administered intradermally to a group of 18 individuals; the response in this group was better than in any of the previous groups. The antigenic potency of the vaccine, therefore, seemed to depend upon its age, increasing age causing diminishing antigenic potency.

In order to test the antigenic differences in other strains of organisms, and also, to compare the effect of the administration of a small dose of vaccine given uniformly by all three methods, a vaccine was prepared from new strains of organisms and administered to a group of 19 individuals. Four received the vaccine intramuscularly, 5 subcutaneously, and 9 intradermally, the dose in all instances being 0.05, 0.1, 0.15, and 0.2 c.c. at weekly intervals. The antibody response (Table II) in these cases was quite marked; evidently small doses of a potent strain are as effective as large doses of the less potent strain. Here again the response after intradermal administration was equally as good, if not better than, by other methods and persisted for just as long a time.

Finally, similar comparative studies carried out on 6 rabbits (Table III) and 12 guinea pigs showed in a general way that the antibody response after

TABLE III

COMPARATIVE AGGLUTININ TITERS AFTER INJECTIONS OF 0.5 C.C. MIXED TYPHOID VACCINE INTRAVENOUSLY, SUBCUTANEOUSLY AND INTRADERMALLY INTO RABBITS

RABBIT NO.	METHOD OF INJECTION	AGGLUTININ TITER			
		BEFORE	3 WK. AFTER	4 WK. AFTER	5 WK. AFTER
1	Intravenously	320	80	640	160
2	Intravenously	320	2580	320	80
3	Subcutaneously	160	160	320	320
4	Subcutaneously	160	320	80	160
5	Intradermally	80	320	1280	320
6	Intradermally	320	320	5120	320

intradermal injection of the vaccine was much better than by other methods. While it is admitted that the presence or the concentration of serum antibodies does not necessarily measure the degree of immunity to typhoid infection, nevertheless, in the absence of statistical evidence such as might be gained from a comparison of the prevalence of the diseases in vaccinated and nonvaccinated individuals during the course of an epidemic of typhoid infection, the agglutination or complement-fixation tests offer our sole means, at present, of determining immunity against typhoid. Since the concentration of antibodies present in the sera of the intradermally injected groups at the end of the six to twelve month period was as good as that present in the sera of individuals receiving the vaccine by other routes and in larger dosage, it seemed apparent that the degree of immunity established by this method was quite as good as by other methods.

The intradermal method of injection was therefore adopted routinely in the prophylactic immunization of individuals against typhoid fever. Four doses, 0.05, 0.1, 0.15 and 0.2 were given at weekly intervals and the vaccine used was not over two months old. While it might be possible to obtain immunity with older vaccines, our experience indicated that a fresher vaccine is preferable. Up until the present, more than 100 individuals have been injected by this method; 87 of these were followed serologically for periods varying between six and twelve months and, as already stated, these examinations have shown that at the end of these periods the degree of immunity was equal to that found by other methods.

The small dosage used and ease of performance of the intradermal injection renders possible rapid administration, thus by using a tuberculin syringe with a single needle, it is possible to give the vaccine to large groups of individuals within a comparatively short time. A striking advantage of the intradermal method is to be found in the marked alteration in the character of the reactions with almost complete elimination of the constitutional reaction. In our cases, following the intradermal injection of the vaccine, there occurred a varying amount of inflammatory local reaction which persisted for a period of two or three hours as a minimum and four days as a maximum, and usually occasioned only slight discomfort. After twenty-four to forty-eight hours in most instances the local reaction had subsided, leaving around the site of injection a gradually fading zone of erythema or at times a small papule which persisted for a somewhat longer period. In a few instances there was slight axillary adenopathy and tenderness, lasting usually about twenty-four hours. Accompanying these local reactions, there were only occasionally such constitutional symptoms as headache, chills, fever, and such gastrointestinal disturbances as nausea, vomiting, and diarrhea, these symptoms being mild in degree in comparison to the reactions occurring after other methods of administration.

TABLE IV

COMPARATIVE SUMMARY OF REACTIONS (GIVEN IN PERCENTAGES) OCCURRING AFTER VARIOUS METHODS OF INJECTION OF TRIPLE-TYPHOID VACCINE*

METHOD	TOTAL OF INJECTIONS	CHARACTER OF LOCAL REACTION				CONSTITUTIONAL SYMPTOMS			
		NEG.	+	++	+++	HEADACHE	FEVER MALAISE	CHILLS	GASTRO-INTES. DISTURB.
Intramuscular	27	7.4	51.8	14.9	25.9	25.9	14.8	11.2	14.8
Subcutaneous	74	15.4	49.0	21.6	14.0	33.6	15.4	8.4	8.4
Intradermal	269	46.2	37.8	12.6	3.4	8.8	2.4	2.8	2.8

*NOTE: Local reactions up to twenty-four hours' duration considered one plus, from twenty-four to seventy-two hours, two plus, and more than seventy-two hours, three plus.

A comparative summary of these reactions is given in Table IV, which contains an analysis of the percentage of reactions occurring after various methods of administration in those persons in whom it was possible to obtain such information after each injection. Two interesting things are brought out in this analysis: (1) the large number of negative or slight (one plus) local reactions after intradermal injection in comparison with the number of reactions after other methods of injections; (2) even more striking, the extremely low percentage of the constitutional symptoms occurring after the intradermal injections as compared with the others. This latter feature constitutes one of the chief advantages of the intradermal method. A logical explanation of the mild character of the reaction would seem to be the small size of the dose, but in those persons receiving either subcutaneously or intramuscularly the same small dose of a potent vaccine as had been used intradermally, the reactions were quite as marked as with the original larger doses. It seems more likely that a slow rate of absorption from the skin occurring after intradermal injection is apt to be responsible for the alteration in the character of the constitutional reaction.

Rather unusual was the occurrence of mild urticarial eruptions in two individuals, one, a member of the subcutaneous group, and the other belonging to the intradermal group. Both of these persons had previously received typhoid vaccine, one as recently as a year. Whether the occurrence of these urticarial reactions may be related to a previous sensitization as has been suggested by other writers,[4] seems not altogether likely in these instances.

SUMMARY

Having demonstrated experimentally that the immunologic response occurring after intradermal injections of small doses of triple typhoid vaccine was equally as good as that produced by larger doses given by the subcutaneous or intramuscular route, I have adopted the following method routinely in the prophylactic immunization of individuals against typhoid fever:

The administration at five- to seven-day intervals of four doses of a freshly prepared (or not more than two months old) triple typhoid vaccine containing one billion typhoid organisms of known antigenic potency and 500 million each of paratyphoid A and B and given by intradermal injection into the upper arm, the first dose being 0.05 c.c., the second 0.1 c.c., the third 0.15 c.c. and the fourth 0.2 c.c. In a series of more than 100 individuals, this method was found to give satisfactory immunologic response, as indicated by the agglutination reaction; to be extremely easy of performance and more important, to produce considerably less local reaction with almost complete elimination of those annoying constitutional symptoms that follow other methods of administration.

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GLUCOSE TOLERANCE IN PERNICIOUS ANEMIA*

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RECENTLY attention has been drawn to the possible relationship of diabetes and pernicious anemia by the occurrence of a small number of cases in which the two diseases were coincident. Root[1] reported 12 cases of the combined diseases. His search of the literature revealed a total of 32 cases. He noted that in nearly all cases where accurate histories were obtained, symptoms of diabetes preceded those of anemia. In diabetes of five years' standing, there usually was a slight anemia, much more marked in patients with achlorhydria. It suggested that diabetes may prepare the way for anemia.[1] Adams[2] reported that at The Mayo Clinic in the five years preceding 1925 there were 1000 cases of pernicious anemia, 2000 cases of diabetes, and only 3 with the combined disease. Giffin and Bowler[3] found evidence that glycosuria, if not true diabetes, may be secondary to anemia in view of a deficit oxygen supply. There is a resemblance between the symptoms of hypoglycemia and the stage of early remission in pernicious anemia, in patients under liver treatment. Riddle[4] has shown that a decrease in the fasting blood-sugar level in pernicious anemia takes place in early remission, probably related to a metabolic adjustment, rather than to a direct effect of liver extract in the blood-sugar level. This relationship suggests that the glucose tolerance in patients with pernicious anemia may vary with the stage of the disease.

This study deals with the glucose tolerance of patients with pernicious anemia, both preceding and following treatment. Determinations of the blood sugar in the fasting state were made by the Folin-Wu micro-blood-sugar method, and determinations made at intervals of one-half hour, one and a half and two and a half hours after the ingestion of 100 grams of glucose in 400 c.c. water flavored with lemon juice.

The results of these experiments are all included in Table I. Of a total of 19 patients with pernicious anemia, the glucose tolerance test was performed before starting treatment in 6 patients; determined during the reticulocyte response in 6 patients (these were all treated with 30-40 grams of desiccated, defatted hog's stomach); and determined after the completion of the "reticulocyte response" in 7 patients. In only one of the nineteen patients was the fasting blood sugar abnormally high; i.e., 143 mg. Eleven of the 19 cases, 58 per cent, showed definitely abnormal tolerance curves, 8 of these having prolonged curves, 3 abnormally high curves. The following standards are taken as normal:

Following a fast of from ten to twelve hours, the normal blood sugar is usually 0.1 per cent, but it may vary between 0.08 and 0.11 per cent. After

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TABLE I

CASE NO.	DISEASE	AGE	SEX	WEIGHT POUNDS	DAY OF TREATMENT	HEMOGLOBIN PER CENT (SAHLI)	RED BLOOD CELLS MILL. PER CU. MM.	WHITE BLOOD CELLS	RETICULOCYTE PER CENT	FASTING STATE		½ HR. AFTER GLUCOSE	1½ HR. AFTER GLUCOSE	2½ HR. AFTER GLUCOSE	
										BLOOD SUGAR MG. PER 100 C.C. BLOOD	SUGAR IN URINE	BLOOD SUGAR MG. PER 100 C.C. BLOOD	BLOOD SUGAR MG. PER 100 C.C. BLOOD	BLOOD SUGAR MG. PER 100 C.C. BLOOD	SUGAR IN URINE
Before Treatment															
1	Pernicious anemia	47	M	131		20	0.78	3200	1.4	121	Neg.	148	173	160	Neg.
2	Pernicious anemia	41	M	125		53	2.37	6550	0.9	91	Neg.	138	182	151	Neg.
3	Pernicious anemia with chronic myocarditis	62	M	188		97	1.94	5500	0.9	143	Neg.	166	181	200	+
4	Pernicious anemia	63	F	114		39	2.16	3250	3.5	117	Neg.	200	148	121	Neg.
5	Pernicious anemia	59	F	85½		17	0.9	3250	2.8	90	Neg.	138	161	171	Neg.
6	Pernicious anemia	70	M	133		70	2.41	4390	1.8	98	Neg.	146	170	147	Neg.
										Av. 110		156	169	160	
*During Treatment**															
7	Pernicious anemia	64	F	130	5	55	2.26	4600	11.0	109	Neg.	177	200	148	Neg.
8	Pernicious anemia	68	M	139½	4	69	2.93	5700	0.4	107	Neg.	137	154	116	Neg.
9	Pernicious anemia	46	M	109½	8	38	1.24	4250	27.8	103	Neg.	172	135	116	Neg.
10	Pernicious anemia	46	M	129½	9	54	2.2	4100	10.4	85	Neg.	147	152	189	Neg.
11	Pernicious anemia	52	M	174	7	66	2.84	7800	10.8	99	Neg.	140	191	142	Neg.
12	Pernicious anemia	42	M	167¼	13	42	1.8	7050	8.5	106	Neg.	200	145	89	Neg.
										Av. 101		162	163	133	
After Treatment															
13	Pernicious anemia	59	F	135		81	3.77	10800	0.9	97	Neg.	132	118	103	Neg.
14	Pernicious anemia	51	M	130½		80	3.93	14200		117	Neg.	178	200	165	Neg.
15	Pernicious anemia	58	M	97		79	3.72	4300		111	Neg.	200	150	135	Neg.
16	Pernicious anemia	59	F	118½		79	4.02	9900		87	Neg.	171	99	86	Neg.
17	Pernicious anemia	24	M	138		59	3.55	9100	4.5	104	Neg.	166	156	110	Neg.
18	Pernicious anemia	62	M	126		75	3.55	12200		104	SPT	145	200	202	+
19	Pernicious anemia	27	M	125		76	3.35	5500		82	Neg.	131	130	105	Neg.
										Av. 100		160	150	129	
										Av. 103		159	164	141	
Controls															
20	Secondary anemia of pregnancy	17	F	92½		64	3.62	8600		101	Neg.	161	142	107	Neg.
21	Aleucemic myelogenous leucemia	67	M	142¼		29	1.57	3650		81	Neg.	146	158	143	Neg.
22	Chronic myelogenous leucemia	22	M	136		50	3.13	224200		47	Neg.	146	137	151	Neg.

*Treated with 30-40 grams ventriculin.

the ingestion of 100 gm. of glucose the average concentration of sugar in the blood is as follows: 0.14 per cent (half an hour); 0.12 per cent (one hour); 0.11 per cent (two hours); and 0.09 per cent (three hours). There is considerable variation from these figures in normal persons. The upper limit of normal is generally regarded as 0.16 per cent, and the curve should return to normal within three hours.

From Table I we see that of 6 patients tested before treatment was begun, 5 had abnormal curves (83 per cent) and 1 had a normal curve. Of 6 patients studied during the "reticulocyte response," 3 had abnormal curves (50 per cent). Of 7 patients tested after the "reticulocyte response" was over or when the blood count was practically normal, 3 had abnormal curves (37 per cent). It would seem that improvement of the blood condition (in these cases induced by treatment with desiccated defatted hog stomach) is accompanied by an increase in the glucose tolerance.

The average curve for the untreated group was 110, 156, 169, 160 mg. per cent. This is a definitely prolonged curve. The average curve for those under treatment is 101, 162, 163, 133 mg. Here the sugar content of the two and a half hour specimen was still slightly high. The average curve for the treated group was practically normal, 100, 160, 150, 129 mg.

The lowest blood-sugar values were found in Case 16 (87, 171, 99, 86 mg. per cent), a patient with a normal red blood cell count. The highest values found were in Cases 3 (143, 166, 181, 200 mg. per cent) and 18 (104, 145, 200, 202 mg. per cent); both patients showed glycosuria at the end of the test. In Case 18, the tolerance was determined after treatment of the anemia; in Case 3, before beginning treatment.

The average curve for the entire group of 19 patients is 103 – 159 – 164 – 141 mg. per cent.

Three controls were included: a patient with secondary anemia of pregnancy (Case 20), aged seventeen years, hemoglobin 64 per cent, red blood cell count 3,620,000, who showed a normal curve (101 – 161 – 142 – 107); a patient with aleucemic myelogenous leucemia (Case 21) male, aged sixty-seven years, hemoglobin 29 per cent, red blood cell count 1,570,000 (81 – 146 – 158 – 143 mg. per cent) with a prolonged curve. A patient with chronic myelogenous leucemia (Case 22), male, aged twenty-two years, hemoglobin 50 per cent, red blood cell count 3,130,000, showed 97, 146, 137, 151 mg. per cent.

An effort was made to correlate the curves with various factors, i.e., with the number of red blood cells, the hemoglobin percentage, the weight, the age, the temperature, and the pulse rates but no gross relationship was evident. In dealing with average figures, it must be borne in mind that one or two abnormally high values might result in the impression of a higher average for the group than individual determinations would warrant. This seems invalidated when over half the total patients showed abnormal determinations.

Again, it must be borne in mind that glucose tolerance as judged by oral administration of 100 gm. of glucose is not necessarily conclusive. It may be that together with disturbed gastrointestinal functioning, these patients with pernicious anemia have a defective absorption of glucose.

CONCLUSIONS

Glucose tolerance studies were made in 19 patients with pernicious anemia. Eleven, or 58 per cent, showed abnormal curves. This abnormality bears no relation to hemoglobin percentage, red blood cell count, weight, age, temperature, or pulse rate.

Six patients were studied before treatment began. Eighty-three per cent of these showed abnormal curves with decreased tolerance. Six patients were studied during their "reticulocyte response." Fifty per cent of these showed abnormal curves with decreased tolerance. Seven were studied after the return of the red blood cell count approximately to normal. Thirty-seven per cent of these showed abnormal curves with decreased tolerance. Improvement of the blood in all of these patients induced by treatment with 30-40 gm. of desiccated, defatted hog stomach was accompanied by increased glucose tolerance.

While the average curve for the entire group shows only slight prolongation over the normal, occasional individual variations were marked.

Further work is necessary to discover whether hog stomach has a direct effect in lowering fasting blood sugar.

The definitely frequent occurrence of disturbed glucose tolerance in pernicious anemia would point to a similarity between certain features of this disease process and diabetes mellitus.

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THE EFFECT OF REPEATED INJECTIONS OF SODIUM ISO-AMYL-ETHYL BARBITURATE ON VARIOUS VISCERA*

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THE widespread use of sodium iso-amyl-ethyl barbiturate (sodium amytal) as a preanesthetic narcotic, as an anesthetic in experimental work on animals and in clinical practice, makes an inquiry as to its effect on the heart, liver, lungs and kidney very pertinent. Although a great amount of physiologic data has been accumulated as to the effect of this drug upon the blood, a study of its effect upon various tissues has never appeared in the literature.

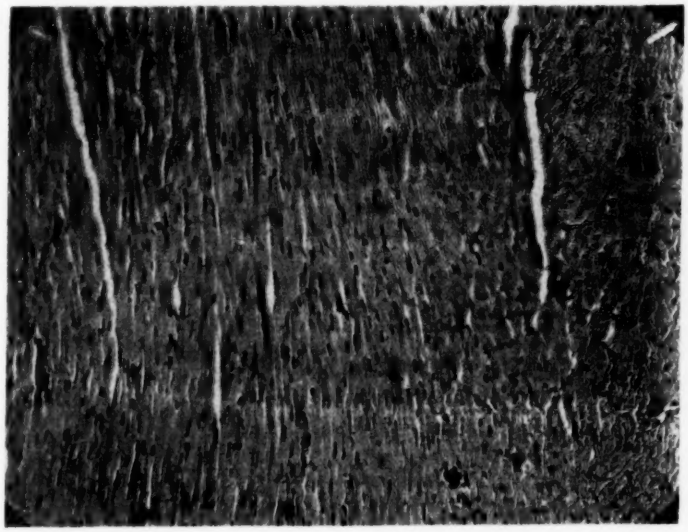

Fig. 1.

This is an interesting commentary upon a substance which has already been administered intravenously to a great many persons for the purpose of analgesia and anesthesia.

The present studies were made on albino rats weighing from 150 to 250 grams. Animals from the same stock (Wistar Institute) which had been on a similar diet for a number of months were used as controls. The latter is essential, since minute alterations in structure often appear in apparently normal control animals. These minute changes might otherwise have been attributed to the sodium iso-amyl-ethyl barbiturate.

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The animals showed no evidence of tolerance to the drug when the same dose was employed on successive days, in that sleep was always induced in the same period of time, three and one-half to four minutes. Table I shows a series of experiments upon a group of 25 rats in which the effect of successive injections of sodium iso-amyl-ethyl barbiturate was studied. All animals in the series were autopsied regardless of the time at which death occurred and specimens were removed from the brain, heart, lungs, liver, and small intestine. These specimens were immediately placed in 4 per cent formalin and after fixing were imbedded in paraffin. Microscopic examination disclosed no evidence of cell injury.* Figs. 1, 2, 3, and 4 are sections from the heart, liver, lung, and kidney, respectively, of animals of this group.

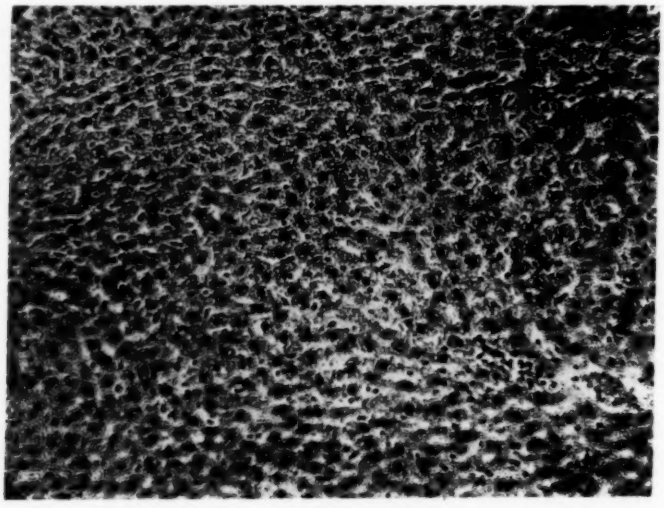

Fig. 2.

TABLE I

NUMBER OF RATS USED	INJECTION	DOSAGE OF SUCCESSIVE INJECTIONS (MG. PER KILO.)	TIME BETWEEN INJECTIONS IN HOURS	REMARKS
25	First	100		12 rats died in 3 hr.
12	Second	50	5	All survived
12	Third	50	18	1 rat died
11	Fourth	50	77	All survived
11	Fifth	50	216	All survived
11	Sixth	75	24	All survived, then killed

In a second series of rats, the effect of varying doses of the drug was studied. Starting with an initial injection of 50 to 150 mg. per kilo of body weight, the amount of the drug injected in this group was varied. The intervals between injections are indicated in Table II. In these animals we were unable to find any microscopic alteration of the viscera studied. Figs. 5, 6, 7, and 8 show sections from the heart, liver, lung and kidney of animals in this group.

*Dr. Joseph McFarland kindly reviewed these slides and confirmed our opinion.

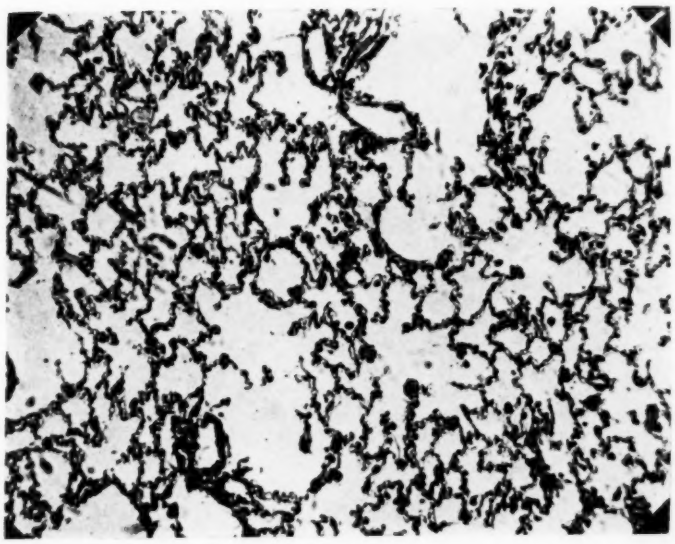

Fig. 3.

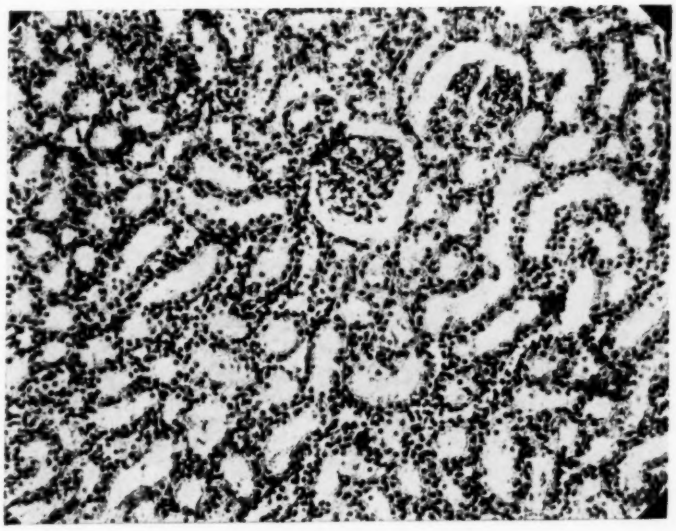

Fig. 4.

Fig. 5.

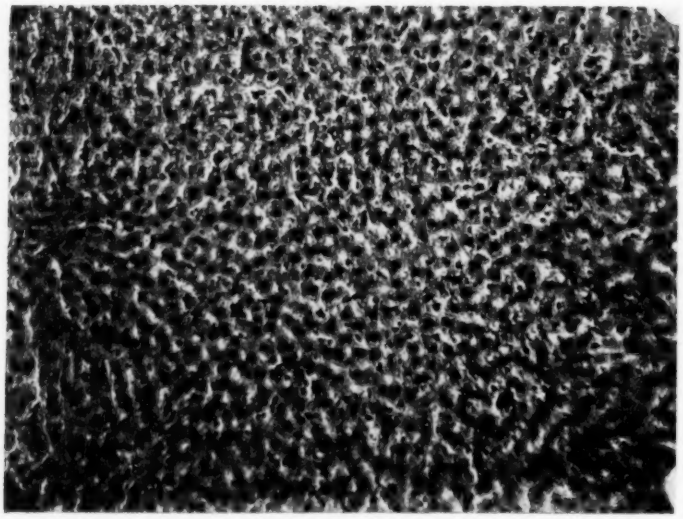

Fig. 6.

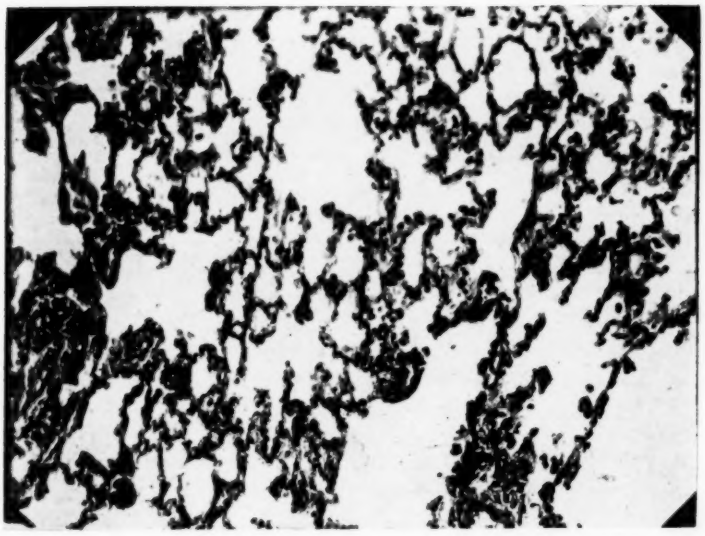

Fig. 7.

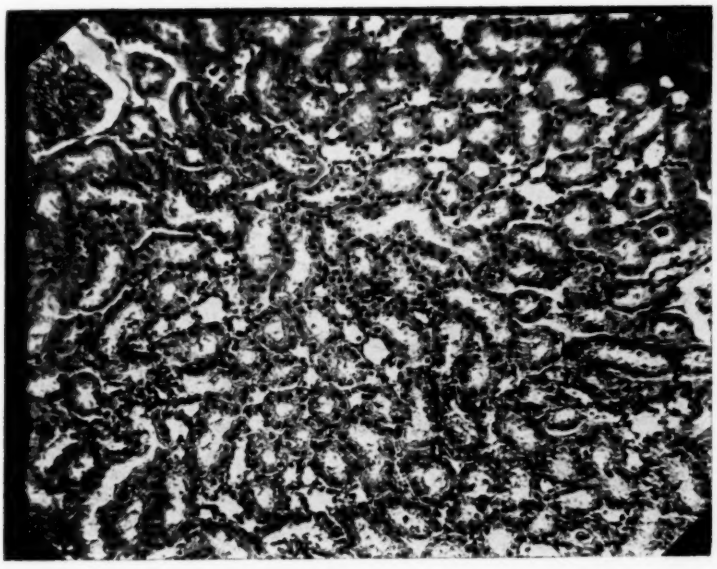

Fig. 8.

TABLE II

NUMBER OF RATS USED	NUMBER OF INJECTIONS	DOSAGE OF SUCCESSIVE INJECTIONS (MG. PER KILO.)	APPROXIMATE TIME BETWEEN INJECTIONS (DAYS)	REMARKS
9	1	50		All survived; then killed
6	1	100		All survived; then killed
3	1	150		All survived; then killed
5	2	100, 150	6	All survived; then killed
1	3	50, 100, 150	2	All survived; then killed
1	3	50, 150, 150	5	All survived; then killed
1	3	75, 150, 150	5	All survived; then killed
1	3	50, 50, 200	2	All survived; then killed
2	3	100, 50, 200	2	All survived; then killed
1	4	50, 100, 150, 200	2	All survived; then killed
1	6	50, 200, 100, 150, 150, 100	7	All survived; then killed
1	8	100, 50, 200, 100, 100, 150, 100, 100	5	All survived; then killed
1	8	50, 100, 150, 200, 150, 150, 100, 100	4	All survived; then killed

Comment.—From this study we are led to conclude that sodium iso-amyl-ethyl barbiturate (sodium amytal) in the amounts used has no effect on the various viscera, which may be affected by various other substances used for anesthesia, as determined by microscopic examination.

A CASE OF LOCALIZED LEUCOSARCOMATOSIS OF THE ASCENDING COLON AND OF THE CECUM*

By Emil Weiss, Chicago, Ill.

LEUCOSARCOMATOSIS has been named by Sternberg,[1] and signifies a condition with a blood picture of lymphatic leucemia and tumors consisting chiefly of lymphocytes, preferably lymphoblasts. In most of the tumors described as leucosarcoma a general involvement of the lymphatic system was observed; a few cases showed a more localized character. Sternberg's classification of these tumors as a special group was opposed by Naegeli,[2] Türck,[3] Herxheimer,[4] Ewing,[5] and others. According to these authors, the majority of these tumors represent only an excessive hyperplasia of the lymphoid tissue as is observed in lymphatic leucemia. Undoubtedly these tumors assume an intermediate position between lymphatic leucemia and lymphosarcoma. A careful study of cases reported as leucosarcoma shows that these tumors form a fluent transition between lymphatic leucemia and lymphosarcoma. Nevertheless for practical reasons the classification of these tumors as a special group appears justified. Mallory applies the term "lymphoblastoma" to all tumors of mesenchymal origin, of which the cells tend to differentiate into lymphocytes. This term would particularly apply to the tumor which is to be described, owing to the fact that this neoplasm consists almost entirely of lymphoblasts.

I think it worth while to report my observation for two particular reasons: (1) There are very few cases of leucosarcomatosis reported, where the intestines are chiefly involved. (2) On account of the localized character of the tumor growth.

REPORT OF THE CASE

Clinical History: A. R., a white male, aged fifty-one, a horseman by occupation, entered the medical ward of St. Ann's Hospital on May 18, 1930, complaining of a large, painful mass in the right iliac and right lumbar regions. He also complained of bleeding from bowels and frequent headaches.

Present Illness: Pain as a result of the rapidly increasing size of the tumor and general weakness caused by the excessive loss of blood are the present complaints.

Past History: The patient did not recollect having any childhood diseases. He was in moderately good health until three years before this illness, when he noticed bleeding from the rectum following bowel movements. The bleeding usually lasted one or two minutes, even when the stools were loose. The patient entered the medical ward of the Frances Willard Hospital on March 16, 1930, complaining of pain in the right side and frequent headaches. The physical examination revealed enlarged lymph nodes in the right axilla and tenderness in the right lower quadrant of the abdomen. The viscera were not palpable.

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The other physical findings were negative. The x-ray examination did not reveal any tumor growth within the stomach or bowels. The urine was negative; the feces contained blood in large quantities on three different examinations. The analysis of the stomach contents showed slight hyperacidity. A blood count made the day after the admission showed 4,024,000 R.B.C., 44,800 W.B.C., with 88 per cent lymphocytes, 4 per cent large mononuclears, and 8 per cent neutrophiles. Two days later the blood count revealed 4,320,000 R.B.C., 54,500 W.B.C., with 88 per cent lymphocytes, 3 per cent large mononuclears, and 9 per cent neutrophiles. Six days later the differential count was 79 per cent lymphocytes, 3 per cent large mononuclears, and 18 per cent neutrophiles. The patient had been discharged on March 29, 1930, feeling fairly well.

Physical Examination: There was a tender firm rounded mass about the size of a grapefruit in the right iliac and right inguinal regions; the region over the liver was also sensitive. The x-ray examination did not show involvement of the extremities or of the chest. The fluoroscopic and the radiographic examinations of the stomach and bowels revealed an obstruction of the hepatic flexure of the colon, indicating malignancy.

Laboratory Examination: Urine and Wassermann tests were negative. The blood count showed on the first examination 4,200,000 R.B.C., 61,100 W.B.C., with 79 per cent lymphocytes, 20 per cent neutrophiles, and 1 per cent eosinophiles; hemoglobin was 65 per cent. Five days later the blood count revealed 4,300,000 R.B.C., 75,600 W.B.C., with 69 per cent lymphocytes, 11 per cent lymphoblasts, and 20 per cent neutrophiles; hemoglobin was 70 per cent. Four days later the blood count was 4,000,000 R.B.C., 46,200 W.B.C., with 66 per cent lymphocytes, 21 per cent lymphoblasts, 13 per cent neutrophiles; hemoglobin was 65 per cent. The last blood count taken two days before his death, was found to be 4,000,000 R.B.C., 73,600 W.B.C., with 64 per cent lymphocytes, 21 per cent lymphoblasts, and 15 per cent neutrophiles; hemoglobin was 65 per cent. The oxidase reaction was negative.

BIOPSY SPECIMEN

On May 22 a laparotomy was performed and the ascending colon, with the cecum and 3 cm. of the ileum, was resected. The patient died ten days later; no autopsy could be secured.

Gross Description: A large nodulated tumor mass involved the major portion of the ascending colon and of the cecum. The tumor infiltrated the wall of the colon, reaching the lumen in several places. Those portions of the tumor which had partially replaced the lumen of the colon did not show any ulceration. The mass was quite solid and homogeneous. The cross-section of the tumor was grayish-white in color and showed occasional small hemorrhages. The diameter of some nodules was as much as 5 to 7 cm. A careful examination of the abdominal cavity during the operation gave the following findings: The liver and spleen were of approximately normal size, the regional mesenteric lymph nodes were moderately enlarged. No tumor growth within the remainder of the bowels could be detected. The results of the exploration of the pelvic region and of the retroperitoneal cavity were also negative.

The Histologic Examination showed a diffuse infiltration of large mononuclear cells, resembling closely the lymphoblasts observed in the blood of this patient. In many of these cells the nuclei contained atypical mitotic figures. Some of the nuclei had 1 or 2 basophilic nucleoli; some nuclei were caryorrhetic. The nuclei were surrounded by a small amount of cytoplasm, which took eosin slightly. A few large and small lymphocytes were found between the lymphoblasts and occasionally a plasma cell. The nuclei of the lymphocytes stained more heavily with hematoxylin than did those of the lymphoblasts. In many areas there were small hemorrhages. The tumor cells appeared arranged in cords, which were separated by small threads of connective tissue, resembling septa. The findings of the portions of the tumor between the nodules were similar. The appendix was also invaded by the tumor cells. The characteristic structures of the wall of the ascending colon and cecum, where the tumor reached the lumen, were completely destroyed and were replaced by the tumor cells. In some areas a few isolated glandular lumina, surrounded by the tumor cells, were the only remnants of the intestinal wall.

DISCUSSION

The blood findings in this case resemble undoubtedly the blood picture of lymphatic leucemia. The clinical history, the physical examination including the x-ray findings, and also the exploratory examination during the operation point to the colon as the main focus of the affliction. The histologic examination of the blood and of the tumor revealed that the tumor consisted chiefly of lymphoblasts, which were also observed in the blood. Sternberg calls a condition, which combines a blood picture of lymphatic leucemia with a tumor consisting of "large mononuclear leucocytes" (lymphoblasts) leucosarcomatosis. This case corresponds to the above definition. Usually in this condition there is a general involvement of organs containing lymphoid tissue; it is seldom that leucosarcomatosis shows a more localized character. This makes the case described so much more interesting.

Leucosarcomatosis can be easily differentiated from pseudoleucemia, leucemia, Hodgkin's disease, and lymphosarcoma. In none of these conditions is there a combination of a constant leucemic blood picture and a tumor consisting chiefly of lymphoblasts. Even without the leucemic blood picture this condition could not be interpreted as pseudoleucemia. In true lymphatic leucemia the hyperplasia of lymphatic tissues is general and never assumes a tumorous character. Hodgkin's disease can be easily eliminated on account of the negative blood picture and different histologic findings in the involved tissues. In lymphosarcoma the blood picture is negative and only occasionally in the terminal stages resembles a leucemic one. Other tumors or tumor-like conditions do not come into consideration.

SUMMARY

A case of leucosarcomatosis has been presented with:

1. A typical blood picture of lymphatic leucemia and a tumor tissue consisting chiefly of lymphoblasts.

2. A localized character involving mainly the ascending colon and the cecum.

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GINGIVITIS IX*

The Rôle of Infection in Gingivitis

By Robert A. Keilty, M.D., Washington, D. C.

THIS is the ninth in a series of papers on gingivitis and I am especially honored to present it before a meeting of the Philadelphia Pathological Society.

For the past several years I have been interested in the pathology and bacteriology of the upper respiratory tract; a more fascinating subject owing to difficulties and variations of opinion could not be found. It has not reached the stage of definitely established basic pathology that other parts of the body have attained and for this reason has great possibilities for advance. The teeth and their supporting structures have been neglected in the past by medical men and the dentists have been mainly concerned with mechanical factors. During the last several years the importance of the teeth in many phases of medicine has been recognized and both professions have made very definite strides out of the maze of fancies and facts.

The rôle of infection has had its share of discussion but due to its many angles and its technical difficulties there are many facets to be yet satisfactorily explained. Its study requires the training and equipment of a bacteriologist but one must also have a good dental experience to cooperate in any study. It is not sufficient to report a hundred smears or a hundred cultures unless the physical conditions under which the study was made are acceptably accurate. As one becomes more familiar with these mouth conditions the influence of associated factors in each individual case is more and more apparent.

There are, however, many conditions commonly found to which little or erroneous importance has been attributed in the past. Many of these points I have tried to bring out in previous papers.[1] For example, the single stained smear report, so-called Vincent's spirochete positive or negative means absolutely nothing and leads to erroneous conclusions unless the type of pathology in the mouth is taken into consideration. The generally accepted view that mouth Amoebae are merely innocuous residents of dirty mouths is erroneous. They are not present in all mouths by any means and are often not found where they might be expected. In addition, they are definitely local pathogens in the sense that while they probably never invade the tissues they keep up a local irritation preparing the ground for invasion by their more vicious neighbors, the streptococci. On the other hand, the Streptococcus viridans, an admittedly dangerous pathogen when it invades a mitral leaflet,

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may be an innocuous resident in the gingival sulcus and not even produce a local reaction. In another case the same cultural streptococcus may produce extensive exudate, necrosis, and false membrane. These differences of reaction depend upon different conditions and thus my statement, the influence of associated factors in each individual case, bears repeating.

There are many hazy points that have become accepted as facts. For example, the so-called Vincent's spirochete best classified under Borrelia vincenti[2] is spoken of in the literature as a strict anaerobe. I have been unable to grow the motile spirilliform types under any anaerobic conditions, I cannot find any literature where this has been accomplished and I have not seen any growing motile cultures in anyone's hands. The Borrelia are probably anaerobic but it is only an assumption that they are.

Based upon this assumption of anaerobiosis substances presumed to liberate oxygen have been advocated and are being used with this end in view. One of these, perborate of sodium, having this extra molecule of oxygen is said to be liberated in some curious way in contact with saliva and to upset the anaerobic conditions for the growth of these spirochetes. This is a widely used drug as a specific in the treatment of all cases of exudative stomatitis, where Vincent's is suspected. At the moment pounds of perborate are being massaged into gums or given in solution when it becomes sodium borate with no more beneficial action than from the alkalinity temporarily set up. Perborate is distinctly irritating in many mouths. Any excess of oxygen liberated would be so rapidly dispersed or combined that it could not possibly influence any anaerobic conditions that might be present. We have seen a large number of cases of acute gingivitis previously treated with perborate of sodium with no effect in the reduction of spirilliform types. We have included the perborate as a part both of treatment and mouth hygiene and after a fair trial have discarded it as of little value.

Under an equally erroneous concept, because it is used intravenously in the treatment of syphilis, arsphenamine is given intravenously in acute trench mouth. Since the value of a specific chemotherapeusis is in the direct contact of the drug with the offending organism, why dilute it through the blood when it can be applied locally to the organism in any desired concentration. As a matter of fact the value of arsphenamine intravenously in acute Vincent's or trench mouth is nil as compared to its value when applied locally. This statement is substantiated by the occasional development of an acute gingivitis during the administration of arsphenamine intravenously for syphilis and by the slight reduction of spirilliform organisms from the gingivae in chronic cases where intravenous treatments are being carried out. In addition, we have had a control series of acute cases where the drug has been previously given intravenously with no effect but where it promptly brings about desired results when administered locally under our concepts of this study of gingivitis.

Realizing the dental phases of the subject such as mechanics, bite, occlusion, poor workmanship on fillings, bridges, crowns, dental calculus and traumatism, I still believe that the bacteria present in a given mouth are the most important etiologic elements in the progression of gingival disease. If the bacteria are given this importance, the various phases of gingival inflamma-

tion become simplified. The question resolves itself into whether or not gingival disease is present, what stage, acute, subacute or chronic, it is in, how far it has advanced and how much permanent bony destruction has resulted. This is easily determined by the history of the case, the age of the patient, the length of the disease, and the pathology present elicited by physical and x-ray examination. It is simple enough from the pathologic conception, but it is hard for the dentist to visualize any similarity between the acute, bleeding, painful exudate of a so-called Vincent's disease and the deep suppurating pockets with recession of gingivae and bony changes of so-called pyorrhea. From the pathologic standpoint of cellular changes, reactions to bacterial invasion, repair, and injury, they are surely one and the same thing. In this conception the whole subject is one of inflammation in the structures about the necks of teeth, of a gingivitis acute or chronic, exudative, necrotic, suppurative, ulcerative, membranous or fibrous. The etiologic factors can be worked out on this basis and are divided into three major groups, dental, somatic, and bacteriologic. We are only concerned with the bacteriologic factors in this paper.

As I have previously stated the bacteriology requires all the experience we are able to command and is preferably to be carried out in a well-equipped laboratory. Great help may be obtained by the dentists treating these conditions, by simple smear examinations but the problem needs a more detailed study. After one has obtained all data possible by complete smear, cultural and animal studies, the interpretation of results must come from combined effort.

In previous papers[2] I have outlined the various organisms as I have found them, including the spirilliform group, Borrelia vincenti; the fusiform group, Fusiformis dentium, Hoelling; the Vibrio sputigenes; the leptothrix group including mycelia, lactobacillus, bacillary rosettes, the fungi and the yeasts. The organisms just mentioned are best seen on smear and with the exception of the fungi and the yeasts are difficult or impossible to culture.

The aerobes, mainly the staphylococcus and streptococcus, are easily obtained on blood agar plates. My experience with these has led to the conclusion that the staphylococcus, several varieties are encountered, is the most common organism, is likely to be the predominating organism, is always present in the acute as well as the chronic forms and is a symbiotic colony in which other types of cocci as well as bacillary forms and fungi may get a start. I have described these colonies in detail. Some form of streptococcus is always present, may be predominating, is present in the chronic as well as the acute cases. When it is present as the predominant it is almost always from an acute case. We may say from the blood agar plates that the predominating organism is playing an important part as an etiologic factor.

The anaerobic cultures are of course more uniform but more difficult to interpret and I would best leave this subject to a future communication.

Little has been said about the yeasts. In previous papers I have merely mentioned them. During the past year I have made further efforts and find that they are rarely seen on smear but by allowing cultures to grow longer, especially on Sabouraud's medium, a much higher percentage than at first

suspected is found. They seem to have a morphology which is not present in yeasts from other sources. The same is true of the fungi. Many mycelial threads seen in smear represent different strains of fungi. When these are cultured on Sabouraud's medium and allowed to grow at room temperature a much higher percentage is found. I believe these will play some part in the chronic cases. I have accidently found that by transplanting some of the heavy staphylococcic colonies from the original blood agar plates eventually a fungus will grow out.

There are many other types of organisms such as B. coli, B. influenceae, pneumococcus, B. diphtheriae, obtained from the cultures but these are peculiar to individual cases and do not repeat very frequently.

The protozoa in the mouth deserve more importance than has been attributed to them. This importance in the case of Amoeba was first pointed out by Allen J. Smith,[3] who fixed its name Endamoeba gingivalis (Gros), and much interest in the subject was initiated by a series of papers by Smith, Middleton and Barrett and by Bass and Johns on this parasite. It is described by Wenyon[4] and has been reviewed by Kofoid and Swezy[5] as well as by others. This Endamoeba is resident in the gingival sulci and in the crypts of tonsils. It has never been described in the gingival tissue but I have recently seen it in large numbers in a tonsil which had all the histologic characteristics of a primary epithelioma but which has not recurred nor metastasized in six months following its removal.

The Endamoeba is easily seen in saline suspension using the high dry objective. In some cases it may be necessary to hunt for it while in others there may be three or four in every field. It is slowly moving as a rule, faster than E. coli but not as precipitate as E. histolytica. It varies considerably in size, may be active or is often found in a resting stage, has a large nucleus, and many vacuoles. The cyst state has never been described. It is usually present in the advanced type of chronic gingivitis with deep pockets, much destruction and suppuration. Very occasionally it is found as the only predominating organism and in these cases treatment with emetine alone clears the case. This is very rare and accounts for the failures in the past and for the fact of its present-day discard. It can easily be eliminated by emetine intramuscularly and if it recurs or reinfection takes place, repeated treatments soon entirely remove it. It has been found that emetine intramuscularly will act just as specifically on mouth Endamoeba as when given locally. We prefer, therefore, the intramuscular use since it is to be given at the same time as local treatments which are complicated enough. We have given or seen given several thousand doses with no bad effect other than occasional sore arms or legs in those who have an idiosynerasy to the drug.

The next most common protozoon is Trichomonas. This is a very active organism, described in detail by Wenyon and present in a relatively large group of cases. It too is found in the chronic advanced cases, is very active, may be found only after searching or may be present one or two to every field. It is never found in the large numbers seen in the feces. It is undoubtedly a pathogen, is very stubborn to eliminate, is not affected by emetine nor by stovarsol locally and the only way we have so far been able to get rid of it

is by hot water irrigations under pressure, in association with local treatment. We have had a few cases of Trichomonas in the feces when present in the mouth but it is not as a rule in both situations. There are other types of protozoa occasionally found but they are not common.

DISCUSSION

In this brief paper I would like to emphasize the fact that bacterial infection and protozoal infestation play a most important part in the initiation and progression of gingival changes. These gingival changes are of real importance since they lead to much suffering, occasionally death in the acute phases and the unnecessary loss of teeth in the chronic phases. The gingivae are a possible focus of infection and this point is almost completely neglected by the medical men. It has been shown experimentally by Cook[6] that elective localization by streptococci from the gingivae is important.

The problem of the bacteriology of gingivitis is very complex and many organisms commonly present have never even been obtained in pure culture. This offers a prolific field for the bacteriologist but conclusions must be controlled by intelligent dental conceptions. The problem as a whole belongs to the dentist and I have found that where a cooperative spirit exists results have been obtained by treatment which cannot be gained by any other method or means so far advanced. The results of treatment based upon these conceptions have been a great aid in evaluating the importance of the rôle of infection in gingivitis.

CONCLUSION

The infectious organisms present in the gingival sulci about the necks of teeth are a most, if not the most important single factor in the etiology of inflammatory conditions from acute to chronic phases, which we have classified under the inclusive term of gingivitis.

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LABORATORY METHODS

TESTS FOR PROTEIN IN URINE, ESPECIALLY BENCE-JONES*

By Edwin E. Osgood, M.D., and Howard D. Haskins, M.D.,
With the Technical Assistance of Mable M. Wilhelm, A.B.,
Portland, Ore.

THE tests for protein in urine in common use will by no means uniformly detect the presence of Bence-Jones' protein. This was first called to our attention in 1923 by the study of the urine of a patient who had been treated for nephritis for some months in a large city hospital. On this day the technician noted a positive nitric acid ring test and a negative heat coagulation test although heat coagulable protein, 4-plus, had been reported previously. We demonstrated the presence of Bence-Jones' protein. Roentgen-ray examination then revealed typical lesions of multiple myeloma, later confirmed by necropsy.

From work with this urine the following facts were established: first, that the concentration of salts and reaction of the urine have to be in certain definite relations to one another to give the typical coagulation at 60° C., complete clearing on boiling, and reprecipitation on cooling which are described in most texts; second, that with certain samples, no precipitate appears at any temperature; and third, that with other relationships the precipitate fails partially or completely to clear, even on prolonged boiling. To overcome these difficulties, the following heat coagulation test was evolved, which will uniformly suggest to the technician the presence of this protein.

To 5 parts of urine in a test tube, add 1 part of 50 per cent acetic acid and 3 parts of saturated (about 30 per cent) sodium chloride solution. Mix and heat to boiling. A precipitate appearing at room temperature on addition of the acetic acid suggests the presence of bile salts, urates, or resin acids. A precipitate appearing at room temperature on the addition of the sodium chloride solution strongly suggests the presence of Bence-Jones' protein. Such a precipitate may also occur in urines containing globulin in amounts of 0.38 gm. per liter or more. Such urines are uncommon. If the precipitate is due to Bence-Jones' protein, it becomes denser on heating to about 40° C. and clears completely (or almost completely) on boiling. Albumin and globulin (in ordinary amounts) coagulate only on heating and show maximum pre-

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cipitation on boiling. The precipitation at room temperature is sufficient to suggest further tests for Bence-Jones' protein. Neither the acetic acid alone nor the salt solution alone in these quantities causes any precipitation of Bence-Jones' protein at room temperature.

In the past seven years this test, which will hereafter be called the *recommended acetic acid and salt test,* has been thoroughly tested in class work and in dispensary and hospital laboratories in several thousand uranalyses. It has proved slightly more sensitive in testing heat coagulable protein than other heat tests. This is particularly true for the urines of low specific gravity containing only traces of protein, which are so common in hypertensive cardiovascular renal disease.

We have had an opportunity to test urines from six other cases of multiple myeloma.* One of these showed no protein of any type in any of many uranalyses up to the time of death. The diagnosis in this case was confirmed by necropsy. The other cases all showed Bence-Jones' protein in large amounts, and in each of these cases it was possible to demonstrate all the previously described reactions. Three of them had had repeated uranalyses in which the Bence-Jones' protein had been missed by experienced technicians using recognized tests for heat coagulable protein, and it was found in these three cases only after the clinical and roentgen-ray signs had established the diagnosis.

In 1927, we began to study the sulphosalicylic acid test for protein in routine uranalysis. We had previously used this only as a test for proteose. The technic which proved most satisfactory is as follows: add 0.1 c.c. (3 drops) of 20 per cent sulphosalicylic acid to 1 c.c. of urine. Boil and cool. Albumin, globulins, proteoses, and Bence-Jones' protein precipitate completely at room temperature. Proteose and Bence-Jones' protein precipitates dissolve completely and quickly on boiling. These precipitates reappear on cooling.† Albumin and globulin precipitates do not dissolve on boiling. This one test, therefore, replaces the Robert's ring test and heat coagulation test previously used and is even more rapid, accurate, and sensitive than either of them alone or than the combination of the two. The clearing of a precipitate on boiling is an indication for doing the recommended acetic acid and salt test. This excludes proteose, which does not precipitate at any temperature with this test.

Experimental Work.—In order to determine the probabilities of detecting Bence-Jones' protein by the various routine tests for protein in urine, the experiments summarized in Table I were carried out.

*Acknowledgment of indebtedness is due Dr. C. H. Manlove of the Good Samaritan Hospital, Portland, Ore., Drs. Norman M. Keith and Carl Greene of The Mayo Clinic, Prof. A. De Castello of the University of Vienna and Dr. Warren C. Hunter of St. Vincent's Hospital, Portland, Ore., each of whom gave permission for the study of the urine from one case. The other two cases were seen in the medical wards of the Multnomah County Hospital, Portland, Oregon.

†Urines containing bile salts, resin acids, or urates give a cloud at room temperature which may completely dissolve on boiling, and reappear on cooling. Concentrations of bile salts sufficient to give this confusing result are uncommon, even when complete obstructive jaundice exists. Precipitation with acetic acid alone and a positive Pettenkofer reaction will confirm a suspicion of their presence.

TABLE I*

PRECIPITATION REACTIONS OF BENCE-JONES' PROTEIN

SP. GR.	P_H	HEAT ONLY NO. 1	HEAT AND ACETIC ACID TEST NO. 2		ACETIC ACID AND NaCl IN SMALL AMOUNTS NO. 3		PURDY'S TEST NO. 4		ACETIC ACID AND NaCl AS RECOMMENDED NO. 5			SULPHOSALICYLIC ACID TEST NO. 6		EXTON'S TEST NO. 7	
		PRECIPITATION	PRECIPITATION		PRECIPITATION		PRECIPITATION		PRECIPITATION			PRECIPITATION		PRECIPITATION	
				BOILING		BOILING		BOILING	RT	40°	BOILING	RT	BOILING	RT	BOILING
1.006	5.1	–	50-60° 3+	–	50-60° 3+	–	40-50° 2+	–	3+	4+	–	4+	–	4+	–
	7.0	–	50-60° 3+	+	RT 50° ± 3+	–	RT 45° + 3+	–	3+	4+	–	4+	–	4+	–
	7.5	–	50-65° 3+	–	RT 55° ± 3+	–	40-50° 2+	±	3+	4+	–	4+	–	4+	–
1.017	5.1	–	55-60° 3+	–	50-60° 3+	–	48-55° 2+	±	3+	4+	±	4+	–	4+	–
	7.0	–	–	–	50-60° 3+	–	45-55° 2+	±	3+	4+	±	4+	–	4+	–
	7.5	+B	–	–	50-60° 3+	–	45-55° 2+	+	3+	4+	–	4+	–	4+	–
1.025	5.1	–	55-60° 3+	–	45-60° 3+	+	RT 50° + 2+	2+†	2+	3+	±	4+	–	4+	–
	7.0	–	–	–	50-60° 3+	+	40-50° 2+	2+†	2+	3+	+	4+	–	4+	–
	7.5	+B	–	–	40-50° 3+	+	40-50° 2+	+	2+	3+	+	4+	–	4+	±

*The change on boiling barely detectable.
†B = precipitation only on reaching boiling temperature.
1+ to 3+ = partial precipitation.
4+ = complete precipitation.
RT = room temperature.
All the precipitates which cleared on boiling reappeared on cooling.

MATERIAL

A urine containing 1.28 gm. per liter of Bence-Jones' protein was diluted with water, normal urine of high specific gravity, and dilute acetic acid in proportions sufficient to give the specific gravities and P_H values recorded in the table. No alkali was needed as the original urine had a P_H of 7.5. It was necessary to add 1/15 volume of saturated sodium chloride solution to get the specific gravity of 1.025. The dilutions were so carried out that the final concentration of Bence-Jones' protein in each sample was 0.64 gm. per liter, just one-half that of the original urine. Thus we had nine samples covering, fairly well, the range of P_H and specific gravity clinically encountered and all containing the same concentration of Bence-Jones' protein.

On each of the nine samples, each of the seven tests listed in the table were performed.

METHODS

Heat Only (No. 1).—Each of the nine samples was heated gradually in a beaker of water with a thermometer in the urine itself. The temperature at which precipitation first appeared (first figure in the table) and the temperature at which maximum precipitation was present (second figure) were observed. Each sample was then boiled over a flame and the amount of precipitate which remained undissolved was recorded. They were allowed to cool to room temperature and any reappearance of precipitate was noted. Any precipitate present was centrifugated out and an excess of 20 per cent sulphosalicylic acid was added to the clear supernatant fluid to determine whether the original precipitation was complete.

Essentially the same procedures were carried out in all the tests so they will not be redescribed for each.

Heat and Acetic Acid Test (No. 2).—To each of the samples used in the preceding test 1/40 volume of 10 per cent acetic acid* was added and the tubes again heated as previously described.

Acetic Acid and Sodium Chloride in Small Amounts (No. 3).—The quantities used were 1/50 volume of 50 per cent acetic acid and 1/50 volume of saturated sodium chloride in 1 volume of the urine mixture. This does not correspond to any commonly used test for protein but was the concentration of salt and acid giving the most typical heat reactions for Bence-Jones' protein with the original urine. It was tried to see if one set of specifications which could be guaranteed to give typical precipitation reactions, could be evolved.

Purdy's Test (No. 4).—The directions usually given are not very specific. We used 1/6 volume of saturated sodium chloride and 1/40 volume of 50 per cent acetic acid.

Recommended Acetic Acid and Salt Test (No. 5).—The quantities, as before stated, are 1/5 volume of 50 per cent acetic acid and 3/5 volume of saturated sodium chloride solution.

*This quantity seemed most nearly to approximate the vague amounts specified in the texts for this test. In our work, all measurements were made with pipettes to be certain that results were entirely comparable.

Sulphosalicylic Acid Test (No. 6).—As previously stated, 1/10 volume of a 20 per cent solution is added.

Exton's Test[1] (No. 7).—An equal volume of reagent (5 per cent sulphosalicylic acid in 20 per cent sodium sulphate solution) and urine are mixed. The directions call for warming gently and state that it is undesirable to boil the mixture, but we carried out the heating as previously described.

RESULTS

Results with ring tests alone are not shown in Table I for it is well known that Bence-Jones' protein gives a positive test with all the ring tests in common use, and hence, would be reported as "albumin" if any of these tests alone were employed.

Note that if heat alone were used, seven of the nine urines would be reported as protein-free, since no precipitate occurred at any temperature, while the two alkaline urines of high and intermediate specific gravity would be reported as containing 1 plus "albumin." If a ring test had been done in addition, as is customary in many laboratories, the seven which failed to precipitate would still probably be considered to contain high concentrations of urates, bile salts, or resin acids (although proteose might also give this result), while the other two would still be reported as containing "albumin."

If the acetic acid were added at boiling temperature, the cloud in the two tubes previously considered to contain "albumin" would clear up and would, therefore, be interpreted as due to phosphates, unless the tubes were saved and examined later, after cooling.

If the acetic acid (No. 2) were added at room temperature and the tubes then heated, four of the nine would be recognized as containing Bence-Jones' protein if they were boiled long enough and the technician were sufficiently observant; a fifth would be regarded as containing both Bence-Jones' protein and heat coagulable protein because of the incomplete solution of the precipitate on boiling. The other four urines would be reported as containing no protein or (if a ring test had also been done) as containing proteose, resin acids, bile salts, or urates. In none was the Bence-Jones' protein completely precipitated by this technic.

The hope that an addition of salt and acetic acid which would give typical results with the original urine would be satisfactory for urines of other P_H values and other specific gravities, proved fruitless as shown by test No. 3. Here, two urines showed slight precipitation at room temperature, six would have been recognized as containing Bence-Jones' protein and the other three might have been reported by a sufficiently observant technician as containing both Bence-Jones' protein and "albumin," but would more likely have been reported as "albumin" alone. Undoubtedly such reactions as those shown in the three urines of high specific gravity have led to the frequent reports in the literature of the association of Bence-Jones' protein and "albumin." Such an association is possible and probably does occur, but it seems probable that most of the cases so reported are based on reactions of the type here shown.

With Purdy's test (No. 4), an even larger number (seven) would have been reported as containing albumin alone or albumin plus Bence-Jones' protein, and only two gave typical reactions. In no case was more than half of the protein actually present precipitated. Hence, even if correctly identified, the amount present would be underestimated. Two urines showed slight precipitation at room temperature.

With the recommended salt and acetic acid test (No. 5), note that all nine urines showed definite precipitation at room temperature, which would at once suggest the probability of the presence of Bence-Jones' protein, and that on boiling, all cleared up completely or almost completely. All but the three with higher specific gravity showed complete precipitation and in these three it was almost complete. Certainly, the presence of Bence-Jones' protein would not have been missed in any of these urines.

The recommended sulphosalicylic acid test (No. 6) gave complete precipitation at room temperature and complete clearing on boiling in the cases of all the urines. Hence, all would have been recognized at once as containing Bence-Jones' protein or proteose and the differentiation could easily have been made by other tests.

Exton's test (No. 7) also gave complete precipitation at room temperature and complete or almost complete clearing on boiling, but clearing did not begin until 70° to 95° C. Therefore, if the orginator's instructions to warm gently had been followed, they would all or nearly all have been reported as containing "albumin" alone. We did not observe the "curdy" character of the precipitate described by Dr. Exton as diagnostic for Bence-Jones' protein until after the tubes had been repeatedly shaken and allowed to stand for some time, and then it could have been overlooked easily. We have observed no advantages of this reagent over the 20 per cent sulphosalicylic acid alone, and since, in the quantities used, it is more expensive and reduces the intensity of the cloud by nearly one-half (dilution), we prefer the 20 per cent sulphosalicylic acid test. Dr. Exton's reagent is, however, very satisfactory if his warning not to boil the urine is consistently disregarded.

To summarize: Ring tests alone will miss all cases of Bence-Jones' proteinuria. Heat alone missed this protein in each of the nine samples studied; heat plus acetic acid added at boiling temperature also missed it in each; heating of the previously acidified urine detected it in four (possibly five) of the nine; Purdy's test detected it in two (possibly seven) of the nine; and Exton's test detected it in none or in all, depending on whether his instructions not to boil were or were not followed. In contrast, the two tests which we recommend detect all and are, if anything, simpler than the other procedures in common use. We prefer the 20 per cent sulphosalicylic acid test for routine use, with the recommended salt and acetic acid test and special tests for Bence-Jones' protein in such cases as show clearing on boiling. Such urines are very uncommon, but very important. When they are discovered, time can be taken to vary the salt concentration and the P_H until the typical reactions occur. These reactions consist of a precipitate appearing at a temperature of 40° to 65° C., and clearing up completely on adequate exposure to temperature varying from 75° to that of the boiling mixtures. This pre-

cipitate reappears on cooling, at 70° to 90° C., and remains at all temperatures below this.* Such a reaction on fresh urine,† coupled with a positive biuret test or other test for protein is adequate evidence that Bence-Jones' protein is present. Other tests are given in reference works. Time can be afforded to identify Bence-Jones' protein if it is suspected to be present, but a simple routine test for protein which will always arouse this suspicion is needed. The tests recommended meet these requirements.

Many other points of interest may be gleaned from a study of Table I, but it is necessary to warn the reader that the effects of small quantities of salt and acid on one urine cannot be transferred to another urine even if the P_H values and specific gravities are the same, since the nature of the electrolytes and nonelectrolytes present also exert complex effects.[3]

Literature.—The observations that the typical behavior of Bence-Jones' protein is dependent on electrolyte and nonelectrolyte (urea, etc.) concentration and on the reaction of the urine, and that variations in these factors can produce extraordinary variations in behavior, is by no means new. Hopkins and Savory,[3] in 1911, made an extremely thorough study of the effects of electrolytes; and before them Magnus-Levy[4] had observed the effects of variations in urea concentration. More recently, Taylor and Miller,[5] Massini,[6] and others have called attention to this point, but examination of ten of the most widely used manuals of laboratory methods (last editions) fails to reveal a single one‡ that clearly recommends a routine test for protein in urine which will uniformly call attention to the presence of Bence-Jones' protein. Three do not mention Bence-Jones' protein at all. Only one gives a satisfactory impression of the varying of the concentration and reaction of the urine which may be necessary before the typical reaction occurs and several mention partial clearing or the association with albumin as not uncommon. Most of the seven texts which mention it, give other tests which are sufficient for its identification if it is suspected. It is obvious that the laboratory will be of greater aid to the clinician if the presence of Bence-Jones' protein can be called to his attention before the clinical findings have led him to suspect its presence.

SUMMARY

1. The routine tests for protein in urine in current use will not uniformly suggest the presence of Bence-Jones' protein.

2. Two simple tests for protein in urine, either of which will call attention to the presence of Bence-Jones' protein, are described.

3. Experiments showing the effects of variations in the concentration and reaction of the urine on the behavior of Bence-Jones' protein to different tests are summarized.

*We mention this last point because at least one laboratory manual erroneously implies that the precipitate redissolves on cooling below 60° C. Once formed, the precipitate is always present, at temperatures lower than the temperature of reappearance on cooling.

†Miller and Sweet[2] have pointed out that in protein-containing urines which have been allowed to stand some time, even if preservative has been added, digestion products of the proteins which give the Bence-Jones' reactions, may accumulate.

‡This statement does not apply to the author's textbook of *Laboratory Diagnosis* which is being published by P. Blakiston's Son and Co., since the results of this study are incorporated therein.

4. The effects of gradual heating to boiling of urine containing Bence-Jones' protein vary with the reaction and concentration of the mixture from no precipitation at all at any temperature, at one extreme, to complete precipitation at room temperature with no clearing on boiling, at the other extreme.

5. The work of Hopkins and Savory and others has not received adequate consideration in the texts on laboratory diagnosis.

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TEST OF HADEN-HAUSSER HEMOGLOBINOMETER

By Joseph Razek,* Ph.D., Philadelphia, Pa.

IN THE Haden-Hausser hemoglobinometer a sample of the acid hematin is placed in a narrow wedge-shaped cell along which a constant thickness of yellow glass is placed. By looking through both the cell and the glass, the point at which the acid hematin matches the glass filter can be noted. The concentration of the solution is determined from the position of the match point. A rather thick blue glass filter placed before a 25 watt lamp provides the light source.

The purpose of this test was (1) to determine how well the yellow glass matches the acid hematin solution as to color, and (2) to determine what the difference in brightness is, when the solution is matched to the glass, in the hemoglobinometer, visually. Since the eye plays such an important part in determining these matches, it is desirable that the test above mentioned be made on an apparatus absolutely independent of any visual determinations.

The test was made with the University of Pennsylvania model of the Razek-Mulder Color Analyzer. This color analyzer is an automatic photoelectric spectrophotometer, by means of which a curve of transmission factor plotted against wave length can be readily obtained. The light to be examined is admitted to a spectroscope in which it is separated into its component colors. A selected portion of the dispersed light is allowed to pass into a photoelectric cell, and the very feeble current from the cell is amplified in a specially developed vacuum tube amplifier. The amplified current is indicated on a sensitive galvanometer, whose reading can be noted on a scale on the front of the machine and at the same time recorded on a photographic film. By turning a small crank, different portions of the spectrum are successively admitted to the photoelectric cell until the entire visible spectrum has been covered, while the photographic film is moved through a corresponding distance. Coordinate lines and an identification number are printed in a separate compartment of the machine. The entire operation of making a spectrophotometric analysis is automatic, and a complete curve can be obtained in about ten seconds.

Since the color analyzer requires samples larger than those used in the hemoglobinometer, the tests were made on specimens of the yellow glass, while the acid hematin was tested in two cells of uniform thickness, 0.0235″ and 0.0335″, and of appropriate area. The acid hematin solutions were of such concentration as to match the yellow glass filter, when placed in the appropriate cell.

On the color analyzer, the usual illuminating head for opaque objects, in

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The Razek-Mulder Color Analyzer. Manufactured by the Burgess-Parr Co., Chicago.

which magnesium carbonate illuminated by a battery of small lamps, is used as a standard, was replaced by a single 200 watt gas filled lamp. Two thicknesses of heavily ground glass were interposed between the lamp and the entrance slit of the spectroscope, to keep the response of the instrument well within the linear range. The advantages to be gained by replacing the magnesium carbonate block by the lamp were (1) greater response for a given change due to the use of a brighter light, and (2) the approximate energy distribution of the lamp is known.

The operation of the tests was as follows: A curve of the light with no test sample interposed in the beam to the apparatus was first made. This gave the standard or 100 per cent transmission. The yellow glass filter was

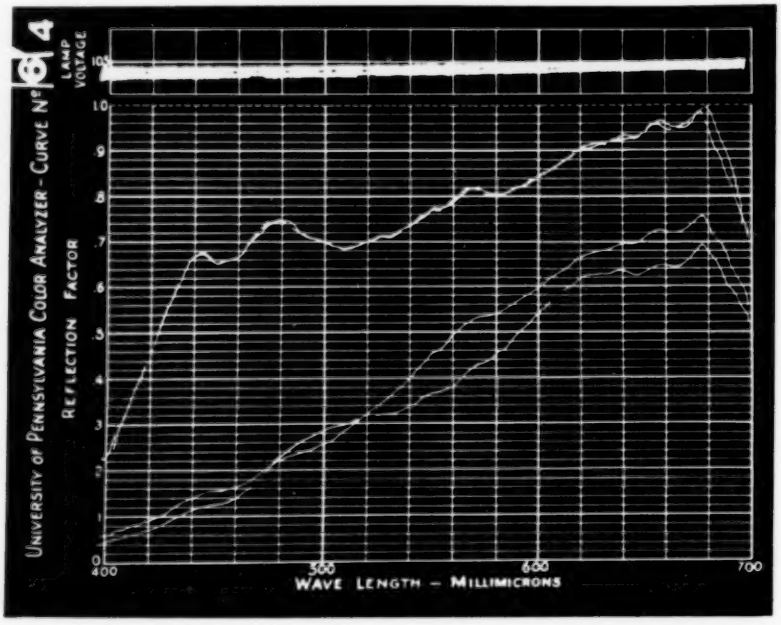

Fig. 1.—Curve No. 64. Yellow glass filter, solid line; 0.0235% acid hematin, dotted line. Original Curves made on Razek-Mulder Color Analyzer.

then interposed in the beam and another curve taken. The same was done with the acid hematin solution in the proper cell. Finally the curve of the light with no sample in the beam was taken again, to check the constancy of the apparatus during the run. To distinguish the curves one or the other was dotted, or broken, as was the second curve on the standard 100 per cent. Each test was run three times and only those curves used on which the standard taken at the end checked with that taken at the beginning of the run to better than 1 per cent. In the run on the blue glass used with the lamp, it was necessary to run the curve at fifteen times normal sensitivity on account of the low transmission factor.

Curves were also taken of the two different acid hematin solutions in their respective cells, to show how nearly the identity of color and hence proportional concentration was realized.

The various curves taken give all the information necessary to compute the desired quantities. However, a curve was also made with the blue glass taken in conjunction with the filter and a cell, which very nearly simulates the optical system of the hemoglobinometer, with the difference that the eye is replaced by the color analyzer.

The representative color analyzer curves from which the subsequent calculations were made are as follows:

Curve No. 64. (Fig. 1.) The upper curve, solid and dotted superposed, is the standard 100 per cent with nothing in the beam. Below it, is found the solid curve, which is the record of the yellow glass filter, and the broken curve, the record of acid hematin in the 0.0235″ cell.

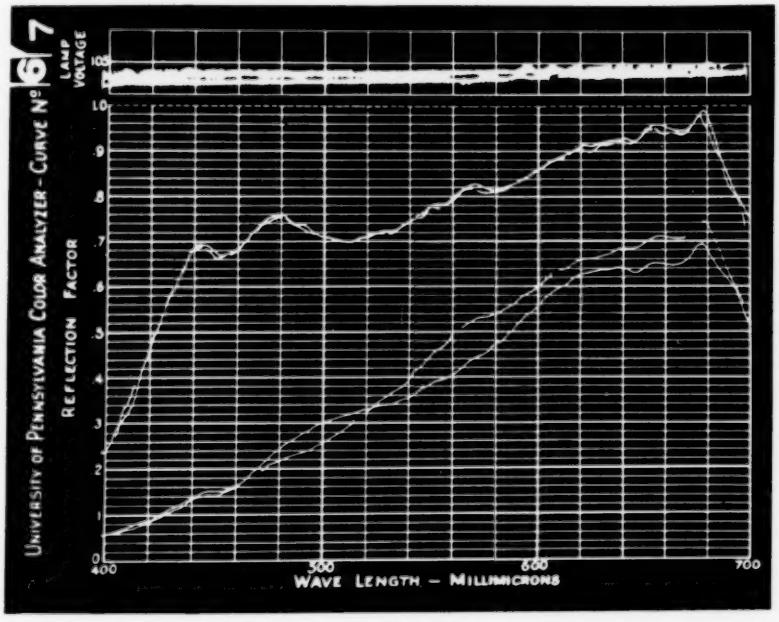

Fig. 2.—Curve No. 67. Yellow glass filter, dotted line; 0.0335″ acid hematin, solid line.

Curve No. 67. (Fig. 2.) This curve is analogous to curve No. 64, except that the filter is shown dotted and the blood, in the 0.0335″ cell, is solid.

Curve No. 53. (Fig. 3.) This is the record of the heavy blue glass filter used in front of the 25 watt bulb of the hemoglobinometer. The top curve is that of the full light. This differs from the other standard curves in that one of the diffusing ground glasses was removed to keep the illumination as large as possible. The lowest curve with a maximum of about 4 per cent, is the transmission through the blue glass filter, referred to the standard. The high curve with a peak at 440 millimicrons is the same curve, with all the ordinates multiplied about 15 times. This makes it possible to obtain the transmission factor with considerable accuracy.

Curve No. 74. (Fig. 4.) This is a record of the two solutions taken in their respective cells. The solid curve is the record of the acid hematin in

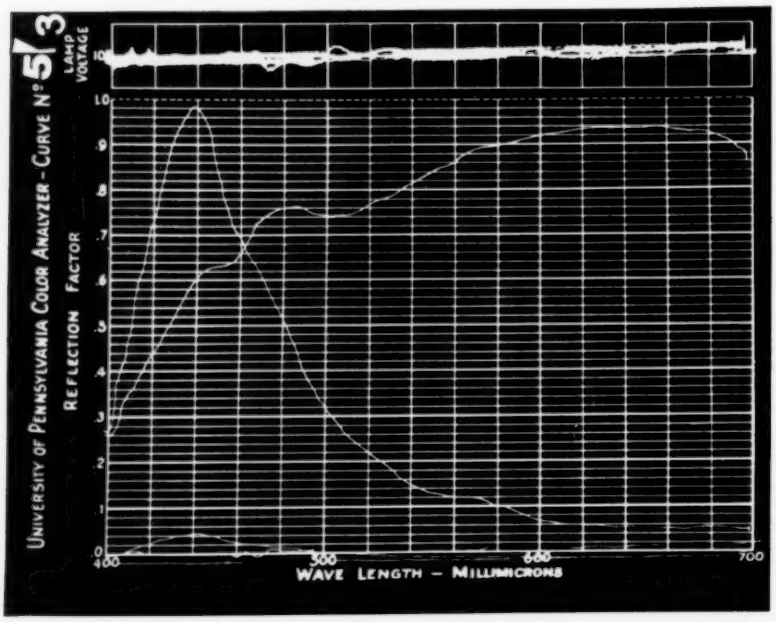

Fig. 3.—Curve No. 53. Blue glass filter.

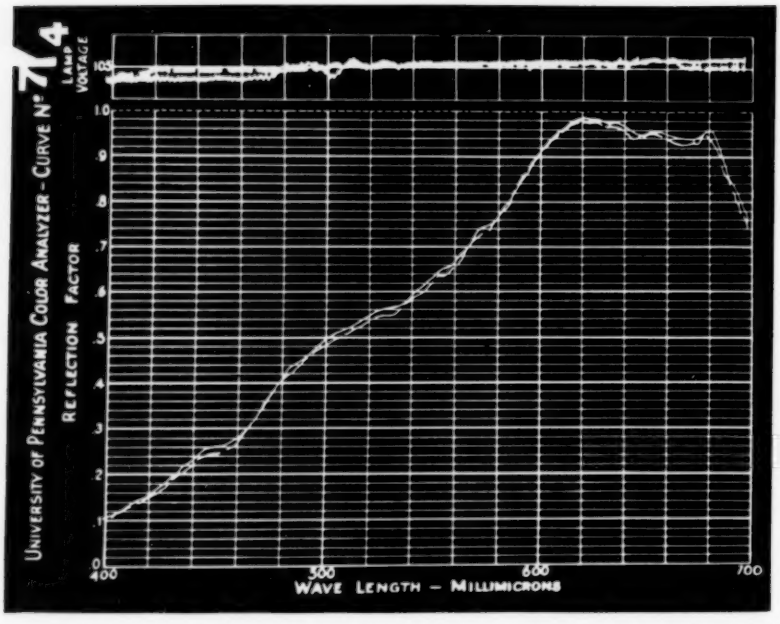

Fig. 4.—Curve No. 74. 0.0335" acid hematin, solid line; 0.0235" acid hematin, dotted line.

the 0.0335″ cell, and the dotted curve the record of the acid hematin in the 0.0235″ cell. This shows a very slightly greater transmission for the former—a fact which is very neatly checked from the other curves.

Curve No. 72. (Fig. 5.) This is a record of the blue glass used in conjunction with the filter for the solid curve, and with the acid hematin for the dotted curve. Since the blue glass filter has such a low transmission coefficient, the light values are much reduced. Consequently the data cannot be obtained from record No. 72 as accurately as it can be computed from the other curves. The general check, however, is sufficiently close, to show the general correctness of the procedure.

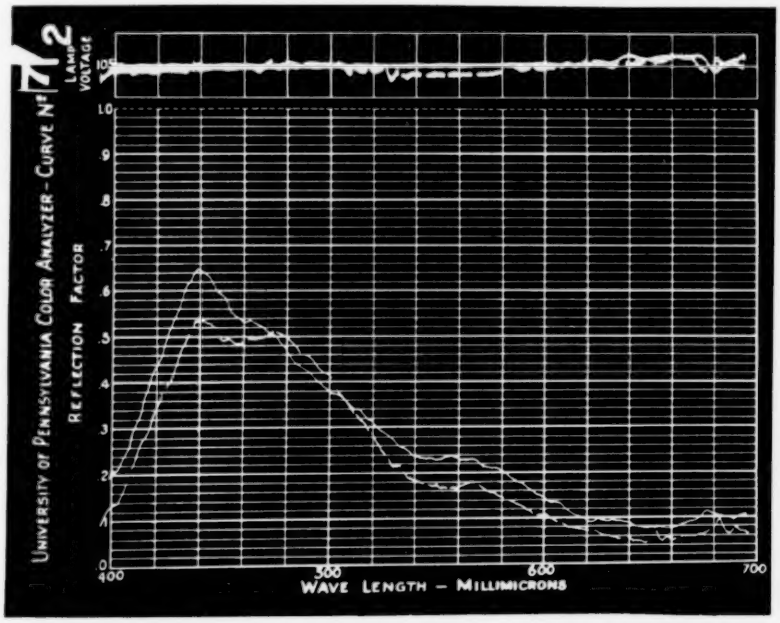

Fig. 5.—Curve No. 72. Blue glass filter + yellow glass filter, solid line; blue glass filter + 0.0235″ acid hematin, dotted line.

The transmission factor for any of the specimens at a given wave length can be obtained by dividing the ordinate for the specimen at the given wave length, by the ordinate for the standard at the same wave length. The results of this calculation are given in columns 2 to 6 inclusive for the various specimens. A plot of one of these is given in Fig. 6. The curve for the other would be so nearly like the one shown that nothing would be gained by plotting it. The transmission curve for the blue glass filter is given in Fig. 7.

The first object of these tests is attained in obtaining the transmission factors. The color match between the acid hematin and the yellow glass filter is quite close, especially when it is noted that the colors of such dissimilar substances, as glass and acid hematin are being matched. Even in the thin cells used, a trace of the familiar absorption bands around 540 millimicrons is apparent.

The second object of these tests was to determine what difference in brightness really exists, when there is an apparent visual match between the solution and the glass filter. Actually, this really amounts to testing the degree of success which the observer who made the match, attained in his efforts.

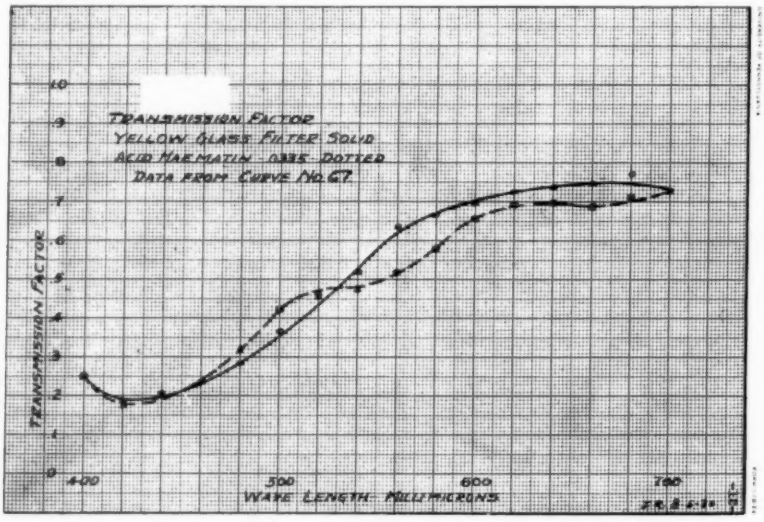

Fig. 6.

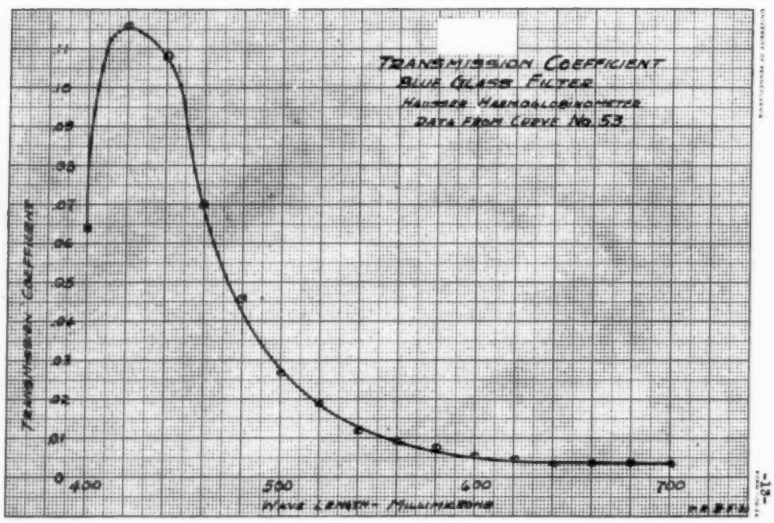

Fig. 7.

Since the eye is used in making these matches, two additional factors must be taken into consideration. The eye is not uniformly sensitive in the various parts of the spectrum. Individuals differ, but a normal "visibility curve" has been adopted by the Optical Society of America, and other interested organizations. The values used in this test are given on page 541 of the re-

port of the Colorimetry Committee of the Optical Society of America, 1920-21. Furthermore, the match is made by looking through the specimens at a 25 watt Mazda lamp, with a dense blue filter interposed. The energy distribution of the light from the 25 watt Mazda lamp therefore enters into the

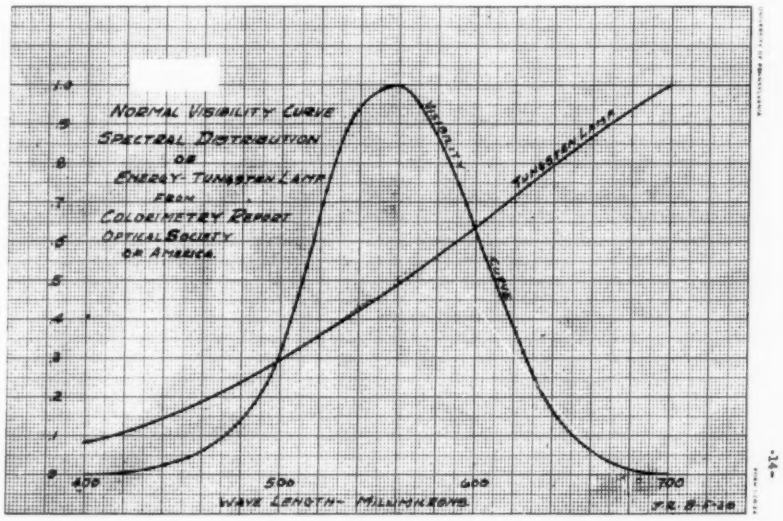

Fig. 8.

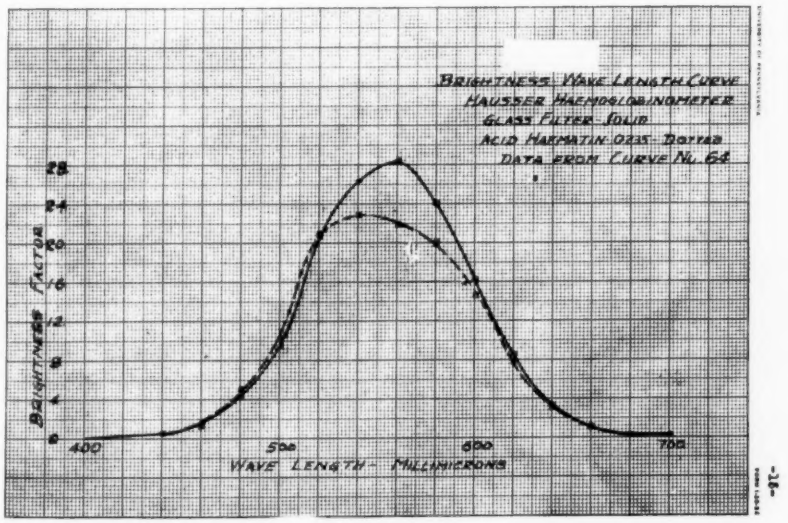

Fig. 9.

final result as noted by the eye. The energy distribution of the light from this particular lamp is not readily available. However, in the Report of the Colorimetry Committee, above quoted, page 561, a curve is shown, of the spectral distribution of energy of a gas filled Mazda lamp, giving the so-called "artificial sunlight." A similar curve for the real tungsten body is

given on page 25 of Zworykin and Wilson, "Photocells and their applications." The curve used in these tests, taken from the colorimetry report together with the visibility curve are plotted on Fig. 8.

With these data, and with the transmission factors as determined from the color analyzer records, it is possible to compute the brightness as seen by the average eye. The brightness factor at a given wave length for a specimen is obtained by taking the products of the transmission factor of the specimen, the transmission factor of the blue glass filler, the ordinate of the visibility curve, and the ordinate of the energy distribution curve of the lamp, all the values being taken at the wave length under consideration. This procedure was repeated for every wave length from 400 to 700 millimicrons, in steps of 20 millimicrons. The results of this calculation are shown in columns 7 to 10 of Table I. Since the quantities are all ratios, the absolute values are of no consequence. The proportionality factor in the brightness factor calculations was chosen to keep the numbers of convenient magnitude.

TABLE I

RESULTS OF SPECTROPHOTOMETRIC TESTS. HAUSSER HEMOGLOBINOMETER

1	2	3	4	5	6	7	8	9	10
WAVE LENGTH MILLIMICRONS	TRANSMISSION FACTORS					BRIGHTNESS FACTORS			
	FILTER	0.0235 BLOOD	FILTER	0.0335 BLOOD	PER CENT BLUE GLASS	FILTER	0.0235 BLOOD	FILTER	0.0335 BLOOD
400	26.1	19.5	25.0	25.0	6.4	0.005	0.004	0.005	0.005
420	20.4	15.8	18.8	17.4	11.0	0.092	0.070	0.085	0.078
440	20.9	17.9	20.3	20.3	10.8	0.75	0.64	0.72	0.72
460	22.9	20.9	23.5	23.5	7.0	1.81	1.66	1.86	1.86
480	29.3	30.7	28.1	32.0	4.6	4.32	4.54	4.16	4.74
500	37.2	40.8	36.7	42.3	2.7	9.45	10.35	9.31	10.75
520	45.6	45.6	45.4	46.6	1.9	20.8	20.8	20.7	21.2
540	54.1	46.0	52.0	47.4	1.2	26.3	22.4	25.3	23.1
560	62.5	48.2	63.4	51.9	0.93	28.4	21.9	28.8	23.6
580	66.6	55.7	66.7	58.0	0.74	24.0	20.0	24.1	20.9
600	71.4	64.4	69.8	65.2	0.56	16.2	14.6	15.8	14.8
620	73.5	69.0	72.5	69.3	0.43	8.46	7.95	8.35	7.95
640	74.7	69.0	73.8	69.6	0.35	3.48	3.21	3.47	3.27
660	75.9	68.4	74.8	68.4	0.35	1.35	1.22	1.33	1.21
680	75.4	70.2	77.0	71.0	0.35	0.40	0.37	0.38	0.35
700	80.0	73.0	72.4	72.4	0.35	0.11	0.10	0.10	0.10
Sum						145.9	129.8	144.5	134.7
Curve	64	64	67	67	53				

The data obtained for the 0.0235″ cell and the filter are plotted in Fig. 9. The integrated brightness as seen by the eye can be considered as proportional to the area under the respective curves. Since the curves are quite symmetrical, a close approximation to the area can be found by merely adding the ordinates given on the result sheet. On this basis, the visual brightness of the 0.0235″ cell is 129.8/145.9 or 89.1 per cent of the glass filter, and the 0.0335″ cell is 134.7/144.5 or 93.0 per cent of the glass filter, as used in the hemoglobinometer.

Referring to Fig. 1, it can be seen that the acid hematin curve is approximately parallel to the curve of the yellow glass filter. If the curve of the latter could be slightly depressed in the range from 500 to 700 millimicrons, so as to cross over the acid hematin curve, at several points, the integrated

brightness of the two would be brought very much closer together. The difference between the two curves the violet and red ends of the spectrum is of less importance because of the low values of visibility at these points. That is, the two curves should be as nearly coincident as possible within the range of 500 to 600 millimicrons, where the sensitivity of the eye is the greatest.

CONCLUSION

While the foregoing procedure may appear arbitrary, it is perfectly definite, and reproducible, and hence can be used to determine the comparison between the two specimens. It is obvious, that whatever interpretation is placed on the results, the desired aim of a perfect match as to brightness and color will be attained, when the transmission factor of the glass filter is the same as the acid hematin solution at all wave lengths.

EXPERIMENTAL ESOPHAGEAL FISTULA*

By Carl A. Dragstedt, M.D., Ph.D., and Ralph B. Mullenix, M.S.,
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SINCE 1895 when Pavlov and Madam Schumova-Simanovskaia[1] demonstrated the relationship of the vagus nerves to the secretion of gastric juice by their sham feeding experiments in dogs having both a fistula of the esophagus in the neck and a gastric fistula, this experiment has been repeated in many physiologic laboratories and is an annual demonstration in some. In Pavlov's description[2] of the technic of preparing dogs with an esophagostomy or esophageal fistula, he states that the operation is simple and usually successful if great care is taken to prevent any soiling of the wound when the esophagus is cut. He stresses the importance of this as the infection in the neck is quite serious and often fatal. He does not mention the mortality experienced in his laboratory.

In our experience the operative preparation of animals with an esophageal fistula has been relatively unsatisfactory. Using the single stage procedure described by Pavlov we have experienced a mortality of approximately 50 per cent in nearly 100 animals over the course of several years. We have not found in the literature any reference to the mortality observed by others in this procedure, but from conversation with workers in several laboratories, we have learned that our experience is not unique and that a high mortality is common. This is well illustrated by a recent paper by Andrus and Donnelly[3] in which they report seven dogs dying from esophageal fistula in from thirty-six to one hundred forty-four hours and apparently concluded that such a condition was incompatible with life and that the loss of saliva played an important rôle in the lethal outcome.

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In the course of some other work that we have been engaged in, it was desired to have a number of animals prepared with an esophageal fistula, and we were anxious to avoid the high mortality previously experienced. In an excellent review of the surgery of the esophagus, Saint[4] has pointed out the reasons for the grave hazard incurred in operations on this organ and has emphasized the desirability of two stage procedures wherever this is possible. Sturgeon[5] and Lahey[6] in recent articles on the operative repair of esophageal diverticulum stress the advisibility of two stage procedures in order to prevent serious and often fatal mediastinitis or cellulitis of the neck. We therefore concluded to use a two stage procedure in preparing the esophageal fistula. At the first operation, a midline incision or one about 2 cm. to the left of the midline is made in the neck, extending for a distance of about 8 to 10 cm., the anterior neck muscles separated and the esophagus delivered into the wound. The esophagus is then carefully freed by gauze dissection from the surrounding fascia, vessels, and nerves for a distance of 6 to 8 cm., and then lifted out of the wound by a hook retractor so that the

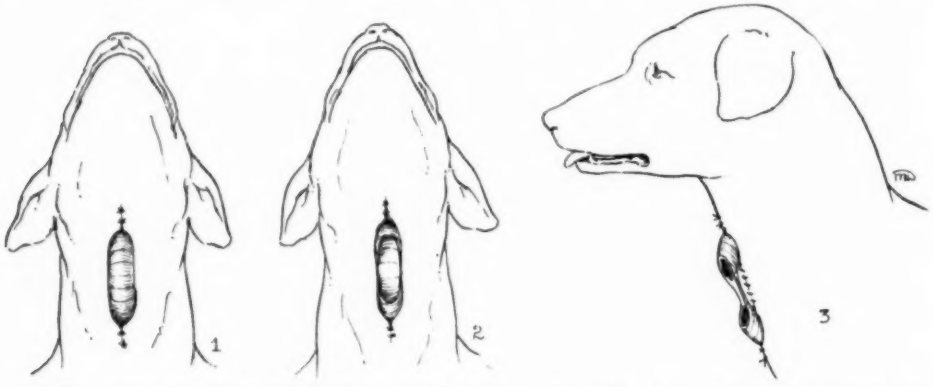

Fig. 1.—A two stage operation for making an esophageal fistula. (*1*), First stage, showing esophagus exteriorized. (*2*), Second stage, performed four or five days after stage 1; showing excision of elliptical segment of anterior esophageal wall. (*3*), Second stage, lateral view.

anterior neck muscles, subcutaneous tissue, and skin can be successively brought together by interrupted sutures beneath the middle part, thus completely exteriorizing a portion of the esophagus about 5 cm. in length. The remainder of the wound is closed and the animal allowed to recover from the anesthetic. If care has been taken in freeing the esophagus from its bed so that no sharp angles are produced, the animals experience no difficulty in swallowing water or food rendered into a thick mush and can be kept any length of time for the second stage. The second stage can be performed at any time after an interval of about four days and consists merely of opening into the exteriorized portion of the esophagus under local anesthesia. We have found it convenient to excise an oval portion of the anterior wall, as illustrated in the drawing, leaving the posterior wall uncut. There is then a double barrelled opening in the esophagus, but owing to the ventral position of the opening and to the slight angularity of the esophagus, no swallowed material will enter the lower segment, while provision is thus made for feeding through such a lower segment if desired as well as for an open-

ing in case vomiting is attempted. If it is desired to close the upper end of the lower segment, this is easily done. The accompanying operations of gastric fistula, Pavlov stomach pouch, or jejunostomy, etc., can be performed either at the first or second stage. We have made esophageal fistulas by this two stage procedure in over twenty animals with no mortality.

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A SIMPLE STAIN FOR METACHROMATIC GRANULES*

By Hardy A. Kemp, M.D., Dallas, Texas

IN BOTH classroom work in Baylor University College of Medicine and in laboratory routine at the University Hospital the following technic for polar body staining has been found to be of advantage over the Alberts and Neisser stains.

METHOD

Smears from the throat or throat cultures are made and fixed in the usual way. The smear is flooded with Gram's iodine for a full minute, rinsed in running tap water, and stained with Loeffler's methylene blue for twenty to thirty seconds. The smear is again rinsed in running water, then stained with saffranine (aqueous 1 per cent) for ten to fifteen seconds. After rinsing the slide again, drying, and mounting, the smear is ready for examination.

By this method, polar bodies are heavily stained a blue-black color while the body of the organism is stained red. Other organisms in the smears from the throat or from throat cultures are stained red. Some, usually the gram-positive cocci, are more heavily stained than others.

It is felt that this stain's advantage lies in the definite contrast of color between the polar bodies and the body of the organism, and in the use of staining reagents which are commonly used in other staining procedures.

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A COMPARATIVE STUDY OF BLOOD PROTEIN PRECIPITANTS*

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THE purpose of this investigation was to compare the effect of certain recently proposed blood protein precipitants on the analysis of the nonprotein nitrogenous constituents of blood. The analysis of the blood filtrate resulting from the precipitation of the blood proteins by the various precipitants was made to determine whether or not any constant variation in the nonprotein nitrogen, urea, uric acid, amino acids, creatine plus creatinine, ergothioneine and tyramine, could be determined.

Table I

CASE NUMBER AND DIAGNOSIS	FILTRATE	NONPROTEIN NITROGEN	UREA NITROGEN	CREATININE + CREATINE	URIC ACID	AMINO ACIDS
1 Nephritic	Folin-Wu	161.3	126.7	5.5	4.2	7.6
	Mo-acid	161.3	123.5	5.7	4.0	8.2
	W-Mo-acid	160.9	122.0	5.3	3.4	9.7
	New Folin	166.7	122.8	5.5	4.2	7.2
2 Normal	Folin-Wu	35.1	11.8	5.6	4.0	7.3
	Mo-acid	35.5	13.4	5.3	3.5	7.4
	W-Mo-acid	33.3	12.1	6.6	3.5	6.7
	New Folin	36.2	13.4	5.3	4.1	6.8
3 Normal	Folin-Wu	35.9	13.7	5.7	2.7	7.3
	Mo-acid	35.4	14.6	5.6	2.8	7.0
	W-Mo-acid	42.1	13.5	5.6	3.0	8.3
	New Folin	39.9	16.3	5.7	3.0	8.4
4 Normal	Folin-Wu	35.6	16.7	5.1	3.8	6.0
	Mo-acid	43.0	16.9	5.4	3.1	7.6
	W-Mo-acid	26.6	17.1	5.3	4.0	6.7
	New Folin	36.4	17.2	4.8	3.8	5.4
5 Eclamptic	Folin-Wu	35.9	16.7	6.1	4.7	6.1
	W-Mo-acid	27.6	15.3	6.3	5.0	6.1
	New Folin	27.9	15.5	6.4	5.3	5.7
6 Normal	Folin-Wu	40.0	----	4.9	3.4	6.1
	W-Mo-acid	33.6	----	4.7	4.4	7.1
	New Folin	38.2	----	4.5	3.5	5.6
7 Tuberculosis	Folin-Wu	34.8	18.3	6.1	4.3	7.0
	Mo-acid	31.6	18.7	6.2	4.4	6.6
	New Folin	29.6	18.6	6.2	4.0	--
8 Eclamptic	Folin-Wu	34.5	20.1	5.8	6.1	8.9
	New Folin	33.8	20.3	5.9	6.7	8.6
9 Eclamptic	Folin-Wu	36.5	17.8	5.6	5.3	8.5
	New Folin	35.4	18.2	5.7	6.0	8.5
10 Nephritic	Folin-Wu	43.7	24.4	6.6	5.7	7.0
	New Folin	44.7	34.7	6.6	5.5	6.1

Blood was drawn by venipuncture, oxalated, and immediately precipitated using the following blood protein precipitants: Folin-Wu's sodium tungstate and sulphuric acid,[1] Benedict's molybdic acid[2] and tungsto-molybdic

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acid[3] reagents, and Folin's new precipitation method involving the nonlaking of the blood.[4] The analysis of the blood filtrate for the various constituents was made according to the following methods: nonprotein nitrogen by the Koch-McMeekin method,[5] uric acid by the method of Benedict,[6] urea by the method of Leiboff,[7] amino acids[8] and creatine plus creatinine[9] by Folin's methods, ergothioneine by the method of Hunter,[10] and tyramine by a modified Hanke and Koessler procedure.[11] Extreme care was taken in making the blood filtrates, and in performing the analyses. It was not possible to run duplicate analyses on account of the large quantity of blood filtrate needed for the various individual analyses.

Table I gives the results of the analyses thus made.

DISCUSSION OF RESULTS

It will be noticed that there is no constant variation in any of the nonprotein nitrogenous constituents in the blood filtrates resulting from the use of various recently suggested blood protein precipitants. With particular reference to the old standard Folin-Wu method, and Folin's new (nonlaking) method, there was no advantage noted for either. In only half of the cases cited was the nonprotein nitrogen content lower with the new Folin method than with the Folin-Wu precipitation procedure. Folin[4] says the new method should give considerably lower results in all cases. In eight of the ten cases the urea nitrogen was higher with the new Folin procedure. The same nonconstant variation was noted in the other results when comparing the new Folin with the older Folin-Wu method. Referring to the results obtained using the Benedict precipitants, one notices the same lack of uniformity in the raising or lowering of the values obtained for any of the blood nonprotein nitrogenous constituents.

Although the table does not indicate the fact, ergothioneine determinations were made on every filtrate. All results were negative. There was not the slightest indication of the pink color described by Hunter[10] as being of quantitative value in estimating the amount of ergothioneine present in human blood. Eclamptic and nephritic bloods were picked for this investigation since it was felt that these two types of pathologic conditions offered the most fertile field for any pronounced variations in the content of the blood nonprotein nitrogenous constituents.

Recent work in this laboratory has shown the presence of tyramine in the blood of eclamptics.[11] Tyramine determinations were run on all filtrates in all the cases shown, normal and pathologic alike. Only in Cases 5, 8, and 9 (the eclamptics) was a trace of tyramine detected. All others showed negative results with the modified Hanke and Koessler method as we used it.[11] In the three cases where the presence of tyramine was indicated, no attempt was made to get quantitative data. The interest in this determination lay in the attempt to determine which of the various blood protein precipitants was best suited to the quantitative determination of tyramine. It was thought entirely possible that the very small quantity of tyramine in eclamptic blood might be lost in the process of preparing the blood filtrates. Therefore, it was our expectation that at least one of the precipitants used might allow a

maximum quantity of tyramine to appear in the filtrate. Accordingly, comparative colorimetric determinations of the intensity of the tyramine color were made on the filtrates from these three cases. No variation in the color intensity could be detected, indicating no appreciable influence of the precipitant on the small quantity of tyramine in the blood of eclamptics.

In conclusion, the authors wish to acknowledge the help of Dr. H. O. Nicholas, who directed this research; and also to acknowledge the assistance of Drs. H. W. Johnson, R. A. Johnston, and Harry E. Braun for supplying the samples of pathologic bloods.

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INDIRECT BLOOD PRESSURE READINGS IN DOGS: DESCRIPTION OF METHOD AND REPORT OF RESULTS*

By Henry W. Ferris, M.D., and John F. Hynes, M.D., New Haven, Conn.

IN ATTEMPTING to show a relation between the experimental reduction of renal volume and blood pressure in dogs, it was necessary to employ some method of pressure determinations which could be used on the unanesthetized animal, without resorting to complicated operative procedures, and which could be used repeatedly over periods of weeks or months, with comparatively accurate results. In the past, many attempts have been made to develop methods in which a cannulized artery might be used for repeated determinations of the pressure, and still other efforts have been directed toward producing apparatus which did not require the use of cannulas. These methods have been reviewed in publications of recent years[1, 2, 3] and need not be repeated here. It is sufficient to say that these have not been satisfactory from several standpoints. Consequently, other investigators have made use of methods more nearly simulating those employed in recording blood pressures in the human being, utilizing a cuff which could be applied to the leg of the animal.

Janeway and Park[4] used a modified Riva-Rocci cuff, one 7.5 × 15 cm. for large dogs, and one 5 × 11 cm. for small dogs. The cuff was wrapped around the lower foreleg. The systolic pressure was obtained by slowly increasing the pressure in the cuff to above blood pressure, and determining the point of obliteration of the radial pulse by palpation. They experienced some difficulty in this determination, especially in smaller animals.

Kolls[1] described an apparatus which consisted of a shell of aluminum so constructed that it would conform to the shape of the external surface of a dog's thigh. The metal was composed of two pieces, in order that it might be adjusted for different sized legs. To this aluminum portion a strip of heavy cloth was attached, equipped with a strap to hold it in place on the inner surface of the animal's leg, the rubber compression bag being placed beneath it. Kolls claimed that with the incompressible outer portion of this cuff, pressure applied through the rubber bag on the internal surface of the leg will cause compression of the femoral artery without losing a component of the force. He had this cuff attached to a recording apparatus, described in detail in his article, by which he was able to take graphic records on a smoked drum.

A blood pressure cuff of the human type, applied as in clinical work, was utilized by Allen,[2] and the pressures determined by auscultation. He employed a cuff of the size ordinarily used for infants, a mercury manometer (Baumanometer), and a stethoscope with a phendoscopic type of bell, about

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2 cm. in diameter. The bell was placed over the small artery at the inner side of the leg just above the ankle and held in place by tapes. The pressure cuff was wrapped about the leg above the ankle. While taking the pressures, the dog was made to stand between the uprights of a frame, and prevented from lying or sitting down, by straps under the belly. He stated, however, that the method was just as applicable to a dog lying on its side. Both Allen's and Kolls's indirect methods were checked by direct intra-arterial pressures, and the results found to be quite closely parallel.

We attempted at first to use Allen's method but the results were unsatisfactory, due to the tendency of the cuff to slip or buckle up, and the difficulty of locating and accurately applying the stethoscope bell to the small vessel found just above the ankle. We consequently tried various modifications of the apparatus, finally developing the one described below.

In any indirect method employing a compression cuff, there are various factors, which have been pointed out by different workers, that may affect the results. McGregor[3] has summarized them as follows: (1) The cuff used should not be too narrow, since part of the pressure is lost in deforming the tissues adjacent to the artery. The size of the cuff must therefore depend on the size of the limb on which it is placed; consequently, a large animal requires a larger bag than a small one, there being more tissue to resist compression. (2) Too loose application of the cuff causes the pressure to appear higher, while too tight application makes it appear lower. (3) The determination of the extravascular pressure is always higher than the intravascular. (4) The point of compression of the vessel should be on a level with the heart. (5) Accurate results are impossible when the surrounding muscles are in a state of contraction.

DESCRIPTION OF APPARATUS AND METHOD

Inasmuch as we were using large animals (18-22 kg.), we chose a blood pressure cuff of the adult size, the rubber bag measuring 13.5 × 22 cm. The strip of cloth ordinarily used to wrap about the arm and hold the bag in place was cut away close to the bag. The latter, by means of its surrounding cloth, was sewed to the central part of a heavy canvas strip which was designed to fit the tapering thigh of a dog. This strip measured 14 cm. wide, 40 cm. long on its upper edge and 26 cm. on its lower (see Fig. 1). It helped greatly in keeping the pressure bag flat against the leg. Two webbing straps (Fig. 1, *c*), about an inch in width were fastened the whole length of this canvas strip, with buckles so placed that the straps could be brought around the animal's leg and the cuff securely held in place. Even with a cuff thus fitted to conform with the shape of the thigh, there was some tendency for it to slip down too far over the knee. Hence, two additional straps were added (Fig. 1, *A*, *B*). The position of these may be best understood from the diagram. The cuff was so applied to the dog's thigh that the rubber tubes leading to the pressure bulb with its valve, and to the manometer, lay on the medial aspect of the leg. On fastening the straps *C* in position by means of their buckles, the point on the canvas to which strap *A* was sewed was brought to lie on the outer aspect of the thigh, between it and the dog board. Strap *A* was then brought around

the body and fastened to its buckle near the groin of the other leg. Strap *B* which was so placed that its origin and insertion were at the same point, passed beneath the body and forward over the body to its buckle near the groin of the blood pressure leg. Of these straps, the latter was found most effective in holding the cuff in place, but the former had a definite value. In using this blood pressure cuff all the straps were pulled up snugly but by no means so tightly as to cause undue pressure on either the leg or the abdomen. The stethoscope bell which was of the phonendoscopic (Bowles) type, with a dia-

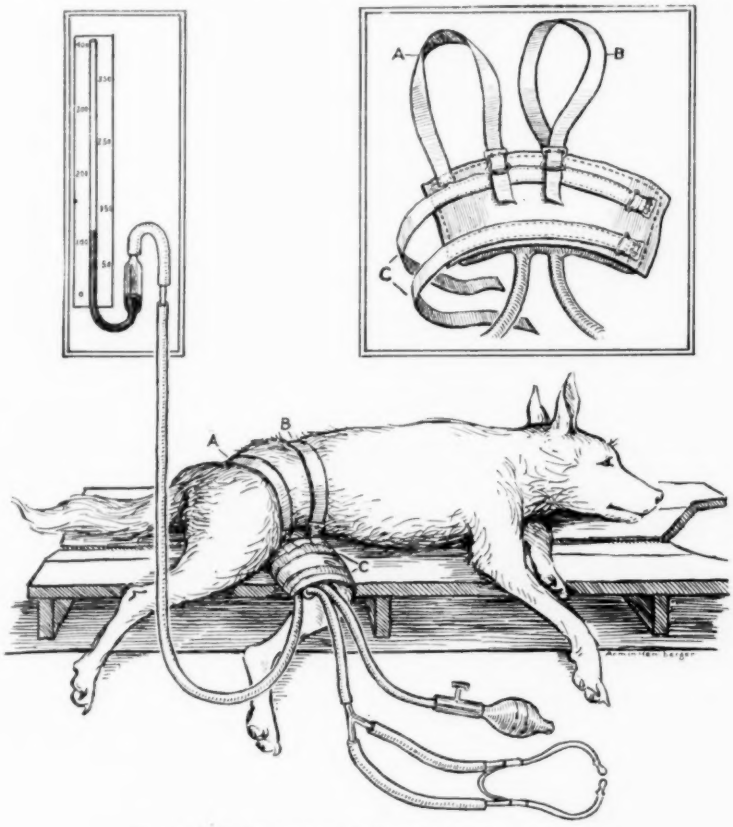

Fig. 1.—Method of applying blood-pressure cuff.

phragm 2.5 cm. in diameter, was placed just beneath the edge of the cuff over the medium-sized artery which could be palpated posterior to the medial epicondyle of the femur. It was not found necessary to tie it in place. The exact position occupied by this bell varied somewhat according to the location of the artery in the different dogs used.

The manometer employed was a mercury one (Baumanometer), of the wall type. We attached it, however, to an upright frame so that it could be stood on a table close by. As seen from the illustration, the left leg was employed for the pressures, the dog lying on that side without being strapped to the board. The hair on the medial aspect of the thigh was clipped closely, thus reducing the adventitious sounds. The systolic pressure was interpreted

as the point at which the pulse sounds just became audible, and was almost invariably quite definite. The diastolic pressure was somewhat more difficult to determine since the sounds tended to fade away gradually in some instances, instead of showing the sudden decrease in intensity which is usually taken as the diastolic pressure. We utilized this criterion when possible, but at times were forced to take the disappearance of sounds as the diastolic pressure. Dogs for blood pressure readings must be chosen which are not too wild or vicious, and can be easily trained. German police dogs are excellent. Females are preferable to males as being somewhat less excitable and likewise better adapted anatomically to the application of the cuff. Some animals cannot be used at all because the pulse sounds are entirely inaudible, or so weak that accuracy in determining them is impossible.

Pressures were taken on the dogs usually about five times a week. Each day an average of at least five readings were made for each animal. If considerable variation occurred, more readings were made until four or five fairly constant values were obtained. The pressure for the day was recorded as the average of five readings. The authors took turns in making the daily records. There was no appreciable difference in the results obtained. This was checked frequently by the workers taking pressures on the dogs together, on the same day. A record was kept of the experimental conditions and such pressures as were obviously altered by factors such as unusual noise in the vicinity of the dog room, entrance in the room of strange persons, and other events which cause an increased excitability of the animals, were discarded in the final results. Muscular contractions of the dog's leg, such as twitchings or a kick, very definitely elevated the pressure, while mental excitation, though the dog appeared physically quiet, likewise would cause elevations of 10-20 points. Among such psychic stimuli were unusual noises and approach of mealtime. The slight physical exertion made by the dog in walking from his cage to the examining room had little lasting effect on the pressures, since we found that by the time we had the dog lying on the table and the apparatus attached, he had quieted down sufficiently so that after the first one or two determinations the readings were fairly constant. Indeed, we found on occasions, that the pressures instead of becoming lower the longer the dog remained on the board, would tend to rise, the dog apparently becoming restless at having to lie quiet so long.

The indirect method described was compared in several animals with direct cannula pressures on the opposite femoral artery. The pressures we found comparable within 5 or 10 mm. of mercury. A rise of pressure was brought about by the injection of adrenalin into a jugular vein. The indirect and direct pressures were found to rise to an equal degree.

The dogs were kept on a definite diet to prevent the loss of weight which would take place if they were fed the routine dog house diet over long periods. That described by Cowgill[5] as Casein Diet No. 3 with additional Vitamin B in the form of Vitavose (Squibbs), plus carbohydrate-rich dog biscuit to complete the caloric requirements, was used. Exercise on a motor driven treadmill was given the animals as regularly as possible for ten to fifteen minutes daily. Blood pressure readings were always taken several hours after this.

and a comparison of the exercise chart with blood pressure records showed no association between pressure changes and the exercise.

EXPERIMENTAL RESULTS

Although our series of animals is small in number (7), we feel that a report of the results is justifiable because of the long periods of time during which blood pressures were taken. Petroff[6] reported studies of the blood pressures of normal dogs taken over periods of time varying from one month to one year, but this method differed from ours.

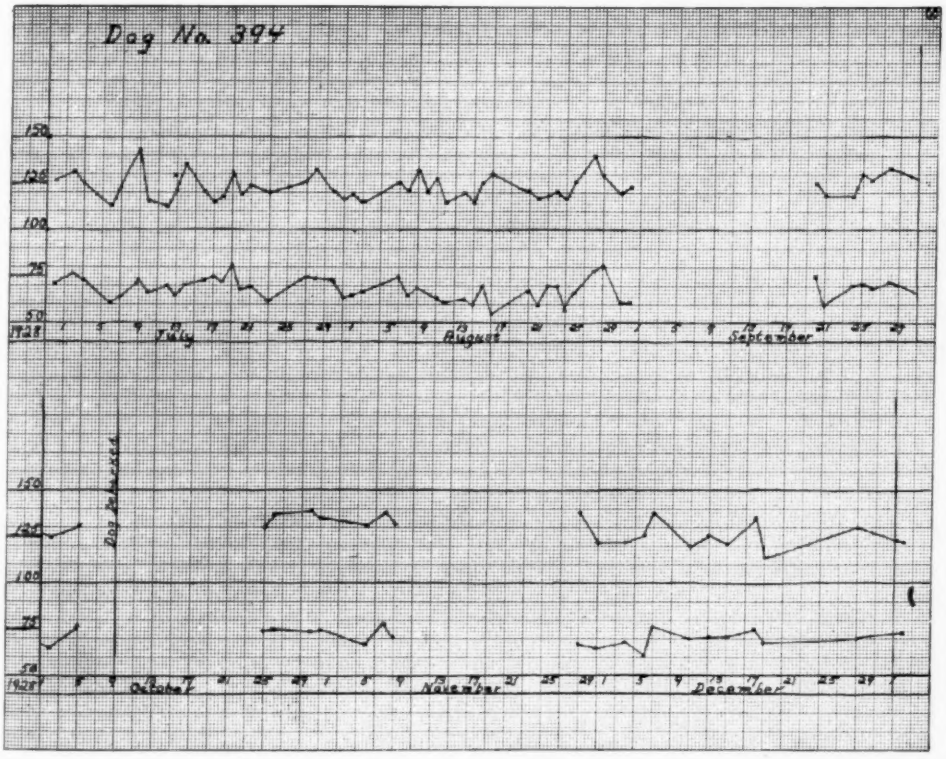

Chart 1.—Experiment I. Blood pressure of dog No. 394, July through December, 1928.

EXPERIMENT I.—Dog No. 394. Mongrel bull, female. The blood pressure of this animal was followed for twelve months, during which time it was taken on one hundred fifty-one days. As is also the case in the following two experiments, six or eight determinations at the beginning of the experiment were not recorded, since at the time the method of taking pressures was still on trial. From Charts 1 and 2 the variations in pressure from day to day may be seen, and likewise the tendency to maintain a fairly constant pressure. The monthly average of the systolic pressure varied from 122 to 137, with a grand average of 130, and of the diastolic pressure, from 65 to 78, with a grand average of 72.

EXPERIMENT II.—Dog No. 439. White collie, male. A total of 161 blood pressure determinations over a period of twelve months were made on this dog (Charts 3 and 4). During the last four months of this period, ergot was administered in an attempt to produce a sustained hypertension. An elevation of pressure was produced, but so many factors were involved, such as diarrhea, loss of weight, and extreme irritability on the part of the dog, that it was thought unwise to draw any conclusions as to the pressor effect of the

ergot used. It will be seen from Chart 4 that there is evidence of the effect of ergot from March to June. The monthly average of the systolic pressure varied from 124 to 146, with a grand average of 136, and of the diastolic, from 72 to 85, with a grand average of 76.

EXPERIMENT III.—Dog No. 454. Brown collie, female. The blood pressure was followed over a period of seventeen months, with a total of readings on one hundred and fifty-three days. For a short time, during March and April, this dog also received ergot hypodermically and the pressures for April reflect the direct or indirect result of this medication. Including this period the monthly average of the systolic pressure varied from 115 to 132, with a grand average of 124; of the diastolic from 72 to 85, with a grand average of 79.

EXPERIMENT IV.—Dog No. 13. German police, female. The blood pressure was taken on thirty-five days over a period of eleven weeks. At the end of this time a left nephrectomy

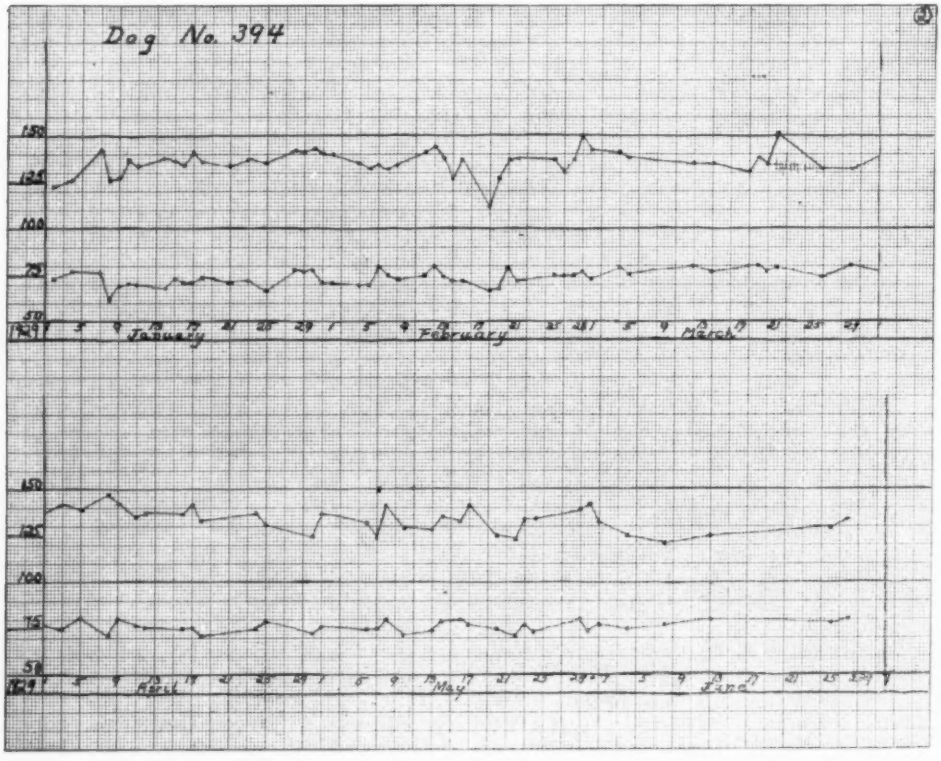

Chart 2.—Experiment I. Blood pressure of dog No. 394, January through June, 1929.

was done. Fifteen days later the dog died of an ulcerative colitis. The first two weeks of pressures on this dog illustrate the fall which is usually characteristic of the "training period" or time it apparently takes the animal to become thoroughly accustomed to the procedure (Chart 8). The average systolic pressure for those weeks was 159 with a diastolic of 102. Exclusive of this period the general average pressure was 123 systolic and 78 diastolic.

EXPERIMENT V.—Dog No. 65. German shepherd mongrel, female. The blood pressure was taken on 36 occasions over a period of fourteen weeks previous to operation and 13 times during three weeks after a left nephrectomy. The dog died at the end of this period under anesthesia given for a second operation. This dog was somewhat difficult to train and its blood pressure remained elevated for a longer time than usual. The earlier pressures do not appear on Chart 8, and may be found in Table I, which is included also to illustrate the method employed for recording data.

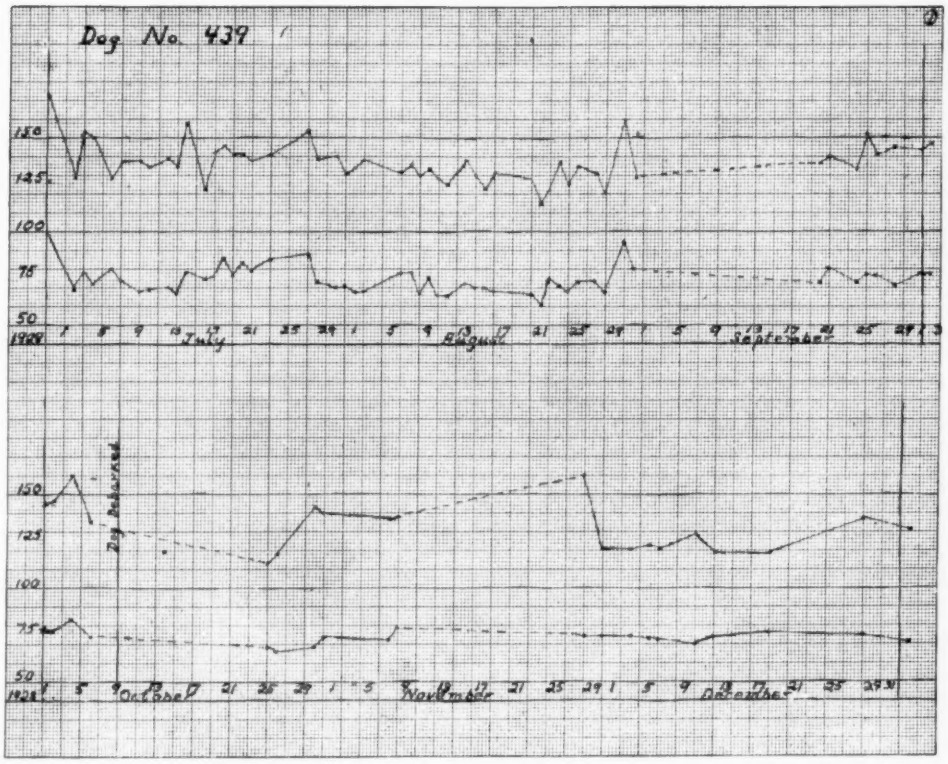

Chart 3.—Experiment II. Blood pressure of dog No. 439, July through December, 1928.

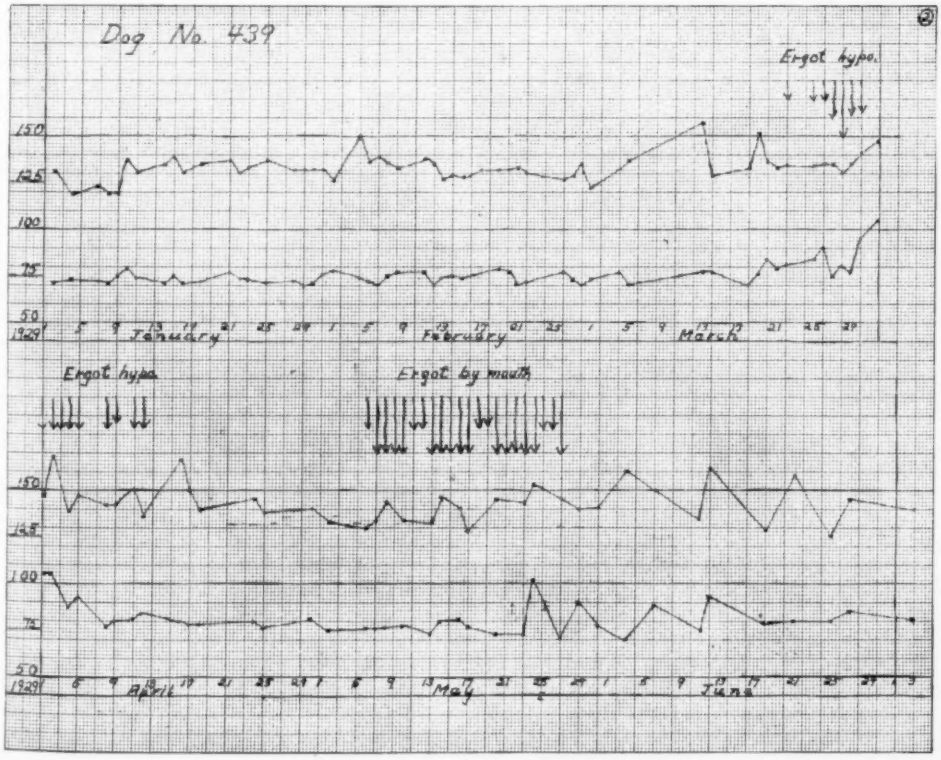

Chart 4.—Experiment II. Blood pressure of dog No. 439 January through June, 1929.

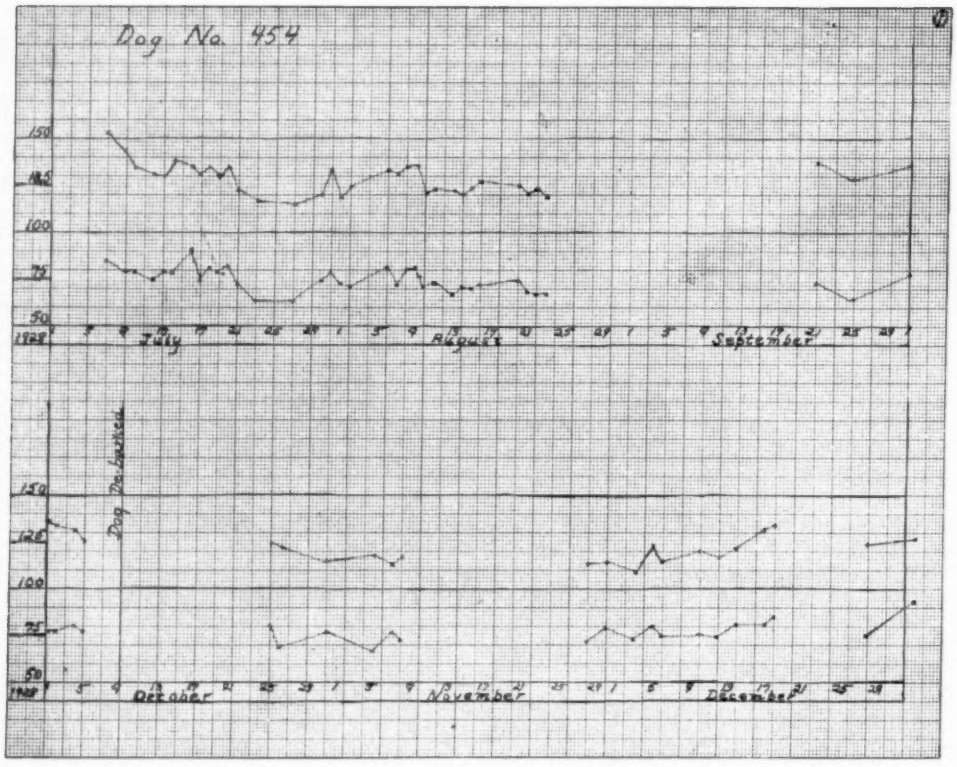

Chart 5.—Experiment III. Blood pressure of dog No. 454, July through December, 1928.

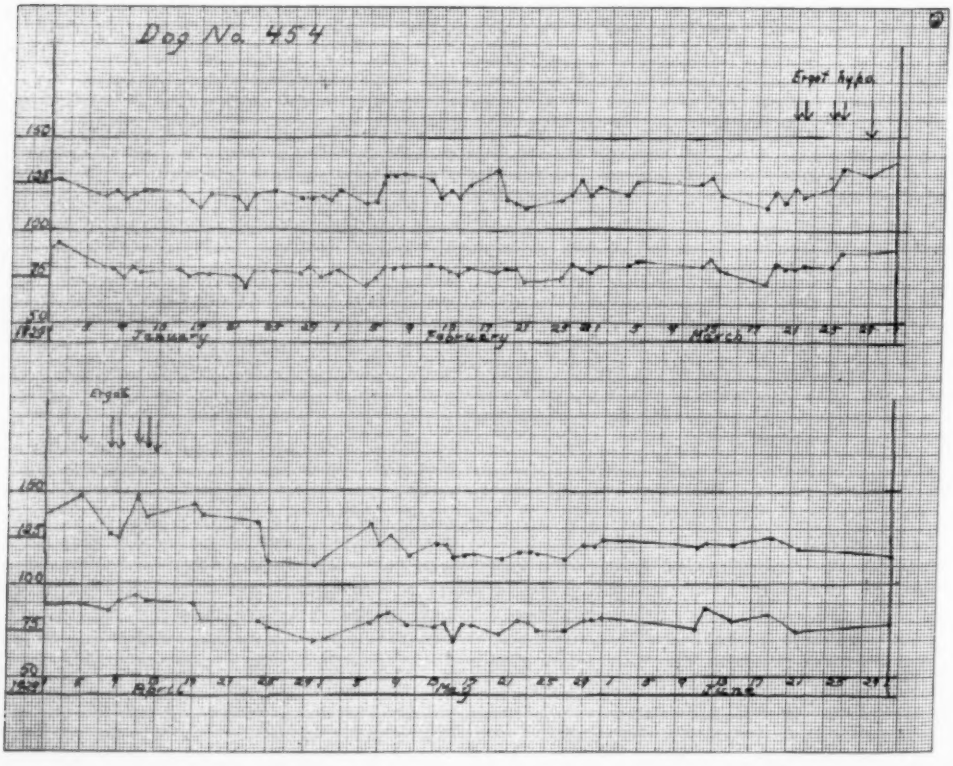

Chart 6.—Experiment III. Blood pressure of dog No. 454, January through June, 1929.

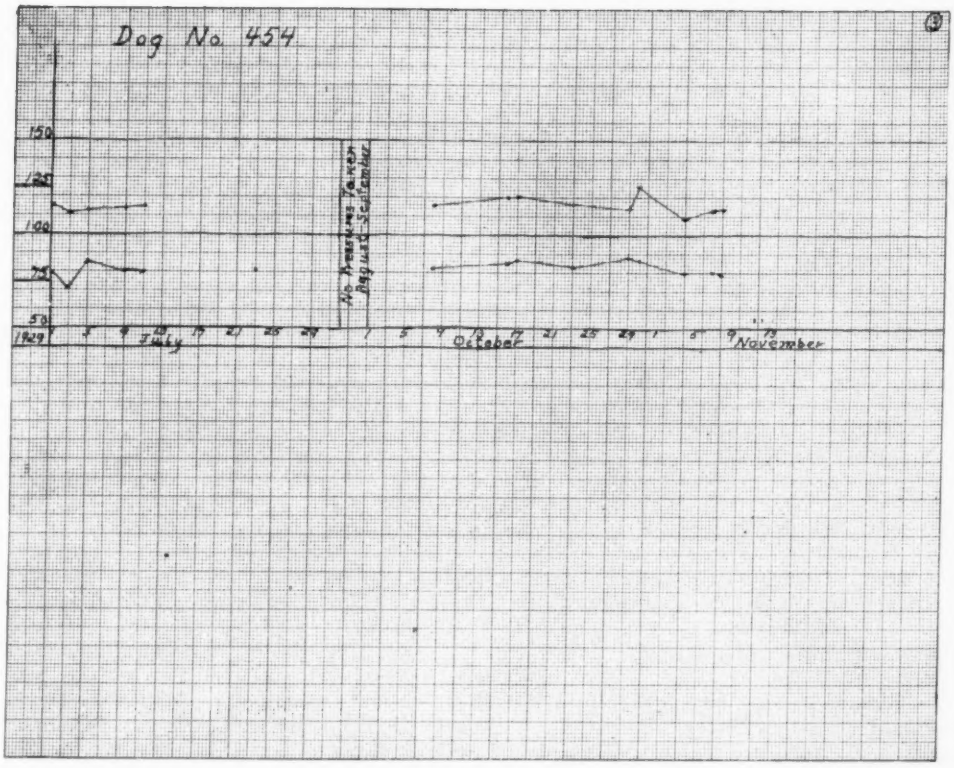

Chart 7.—Experiment III. Blood pressure of dog No. 454, July through November, 1929.

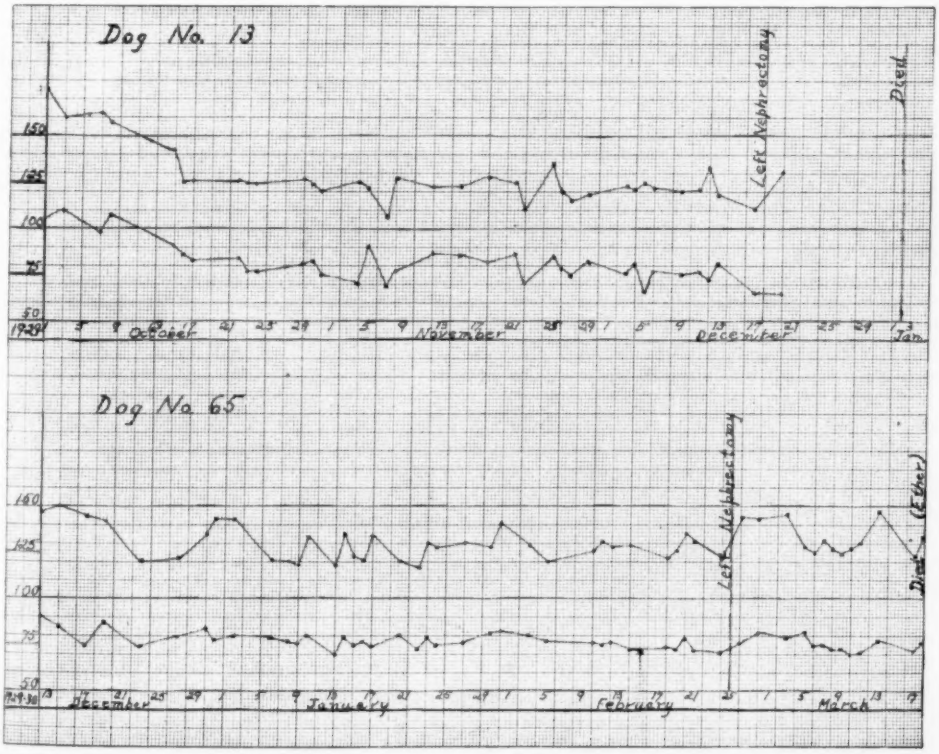

Chart 8.—Experiments IV and V. Blood pressure of dog No. 13, October through December, 1929, and of dog No. 65, December, 1929 to March, 1930.

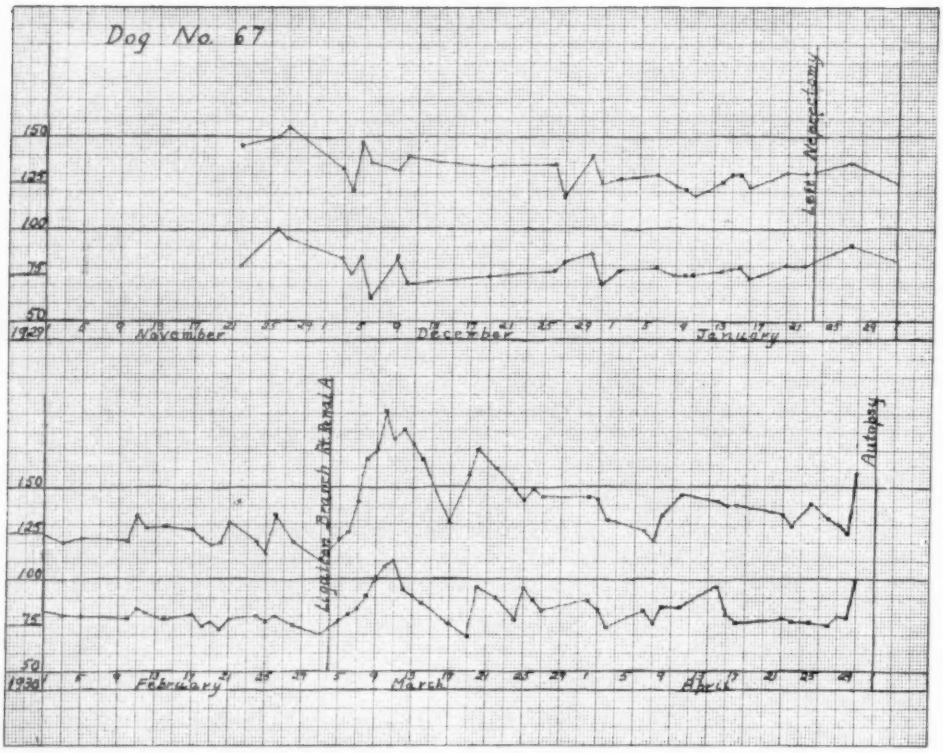

Chart 9.—Experiment VI. Blood pressure of dog No. 67, November, 1929 through April, 1930.

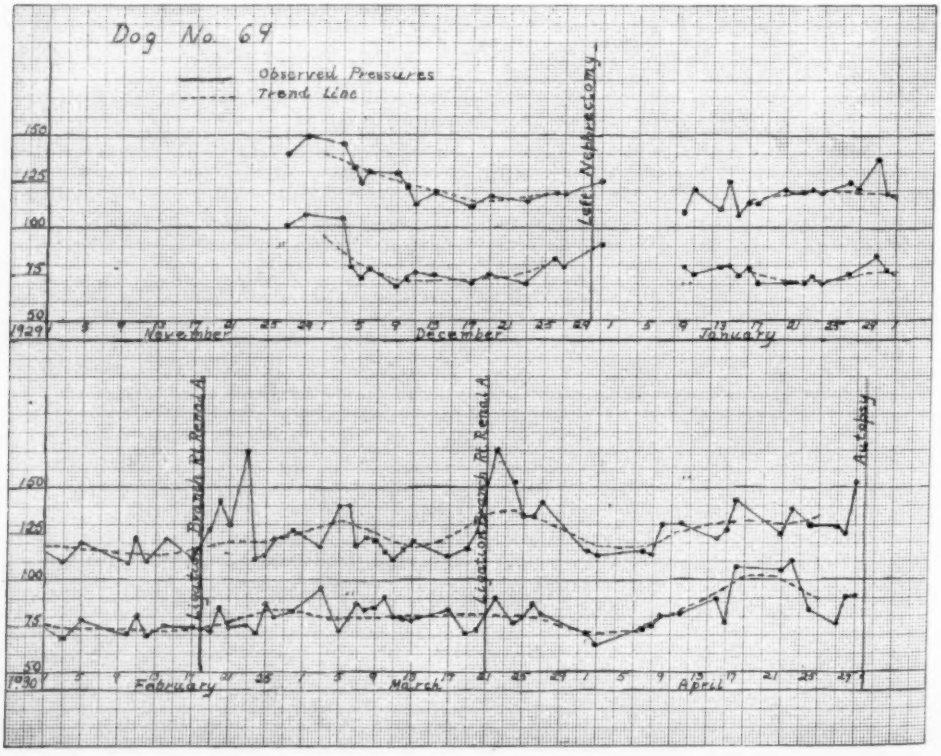

Chart 10.—Experiment VII. Blood pressure of dog No. 69, November, 1929 through April, 1930.

TABLE I

DOG 65. PROTOCOL OF EXPERIMENT V. NOVEMBER 18 TO DECEMBER 17

DATE	INDIVIDUAL READINGS	AVERAGE PRESSURE	EXPERIMENTAL CONDITIONS AND REMARKS
11/18/29	None		No pressures taken; trained to lie on board.
11/21/29	140 90	?	Dog quiet but shivering; sounds hard to hear.
11/22/29	145-145 80-85	145/83	Restless at first, quiet later. Dog house noisy. Sounds good.
11/25/29	125-135-135 85-85-90	131/87	Quiet at first, later restless. House fairly quiet. Sounds fair.
11/26/29	None		House very noisy, sounds inaudible.
11/27/29	155-165-140-150 100-85-80-80	150/87	Dog restless; house quiet; sounds clear.
11/29/29	135-125-120-120 80-80-75-70	125/77	Dog quiet; house noisy; sounds faint.
12/ 3/29	155-150-160-150-150 70-80-65-80-80	153/75	Dog restless; house quiet; sounds clear.
12/ 4/29	135-145-140-145-130-135 80-80-75-80-80-75	137/79	Dog quiet at times; at others restless, house quiet, sounds clear.
12/ 5/29	130-135-130-130-140 85-85-85-90-90	133/87	Dog restless; house fair; sounds clear.
19/ 9/29	120-120-125-120-120 80-80-80-70-70	121/78	Dog quiet; house quiet; sounds clear.
12/11/29	140-140-145-145-145 80-75-80-85-80	143/80	Dog very restless; house noisy; sounds fair.
12/12/29	160-150-145-140-140 90-90-90-90-90	147/90	Dog restless; house fair; sounds fair.
12/13/29	145-135-145-165-160 80-80-75-90-100		Dog so very restless that pressure readings all obviously without value.
12/17/29	145-145-145-145-145 75-75-75-75-75	145/75	Dog fair, house fair; sounds clear.

As will be seen from the above protocol, there was great difficulty in training this animal to lie quietly while pressures were being taken. Table I should be contrasted with Table II (Dog No. 69).

EXPERIMENT VI.—Dog No. 67. Airedale, female. Blood pressure determinations were made on this animal over a period of twenty-two weeks. On January 23, 1930, a left nephrectomy was performed, following which the blood pressure remained practically unchanged for five weeks. On March 4, a large branch of the right renal artery was ligated. A marked rise in pressure occurred shortly after the operation, as may be seen from Chart 9, but was not sustained. After four weeks the pressure had fallen considerably, but it maintained a level somewhat higher (10 mm. systolic) than the pre-operative level, for the following four weeks. The average pressure before operation was 132/79, after the first operation 124/79, after the second operation, 146/85.

EXPERIMENT VII.—Dog No. 69. German police, female. The blood pressure was followed for twenty-two weeks on this dog. There was a rapid initial fall of the pressure, reaching a level which it maintained for three weeks, when a left nephrectomy was performed. There was no noticeable change for seven weeks, and on February 18 a large branch of the right renal artery was ligated (see Chart 10). Save for a marked rise (to 170 mm. systolic) on one day, the blood pressure was little affected. Four and one-half weeks later, another branch of the right renal artery was ligated. The rise in pressure after this operation lasted about a week, when the pressure fell to normal. This was followed by a secondary rise, and the dog maintained a systolic pressure 10-15 mm. above normal for the last three weeks before he was sacrificed. A trend line was calculated for the blood pressure graph of this animal, using the formula $P = \frac{a+2b+c}{4}$, in which P is the point on the

trend line, *b* is the pressure on the corresponding day, *a* the pressure four days previously, and *c* the pressure four days later (consult Chart 10). The average pressure before ligation of a vessel was 121/78; after both ligation operations, 132/85.

TABLE II

DOG NO. 69. PROTOCOL OF EXPERIMENT VII. FEB. 1 TO MAR. 1

DATE	INDIVIDUAL READINGS	AVERAGE PRESSURE	EXPERIMENTAL CONDITIONS AND REMARKS
2/ 3/30	120-105-110-110-105 75-65-70-65-65	110/68	Dog quiet; dog house noisy. Diastolic pressure hard to hear.
2/ 5/30	115-115-120-125-125 80-75-75-80-80	120/78	Dog quiet. House noisy. Sounds fairly clear.
2/10/30	110-105-110-110 70-70-70-70	109/70	Dog quiet. House noisy; sounds clear.
2/11/30	125-125-125-120-120 80-80-80-80-80	122/80	Dog quiet. House fair. Sounds clear.
2/12/30	110-110-110-110-110 70-70-70-70-70	110/70	Dog quiet; house fair. Sounds faint.
2/14/30	120-120-125-125-120 80-80-70-75-70	122/75	Dog quiet. House quiet. Sounds clear.
2/17/30	110-110-110-115-115 75-75-70-75-75	112/74	Dog restless at times. House noisy. Sounds fairly clear.
2/18/30			Artery to lower pole of right kidney doubly ligated through abdominal incision.
2/19/30	130-130-125-130-125 75-70-75-70-70	128/72	Dog quiet. House fair. Sounds clear. Wound clean.
2/20/30	140-145-145-145-140 90-85-85-80-85	143/85	Dog quiet. House quiet. Sounds clear. Some edema of wound.
2/21/30	130-130-130-130-130 75-75-75-75-75	130/75	Dog very quiet. House very quiet. Sounds very clear. Dog in good condition. Wound edematous and oozes serosanguineous fluid from anterior end.
2/23/30	170-170-170-170-170 75-75-75-75-80	170/76	Dog quiet. House quiet. Sounds very loud and clear. Wound the same.
2/24/30	110-115-110-115-110 70-70-70-75-70	112/71	Dog quiet. House noisy. Sounds faint probably taken too low; wound still drains slightly.
2/25/30	115-110-115-115-115 90-90-85-90-80	114/87	Dog very quiet. House very quiet. Sounds very faint, especially diastolic. Systolic by palpation about 110.
2/26/30	125-120-120-120-125 80-80-80-80-80	122/80	Dog quiet. House quiet. Sounds clear. Palpation of systolic, 115-120. Wound healing well.
2/28/30	120-125-130-125-135 80-75-85-85-85	127/83	Dog restless. House noisy. Sounds loud and clear. Wound dry. Edges well apposed.

Compare with Table I, and Chart 10.

DISCUSSION

The data which are here reported were obtained during a series of experiments on the effect of reduction of renal substance on the blood pressure. The study of this problem is still in progress, and we are presenting here, therefore, merely a preliminary report on the methods employed. We believe that in the greater part of the previous work on experimental alteration of blood pressure, controls on the methods employed in demonstrating that alteration have not been carefully maintained. In this connection it may be pointed out that absolute accuracy in determining the pressure is of less importance than the ability to follow relative changes, when the problem of experimentally altering the blood pressure is involved (Allen). The objection

which we would make is not so much against the technic employed in indirect measurement methods, as against the failure of some investigators to recognize certain characteristics of the graph obtained by plotting the blood pressure over a period of weeks or months.

As far as we have been able to ascertain from the literature, the possibility of seasonal or cyclic variations in the dog's blood pressure has never been determined. From our observations on Dogs 394, 439, and 454 (Experiments I to III) we believe that there are no such variations of significance, and our belief has been further confirmed by the remaining experiments (IV to VII). On the other hand, a fall in blood pressure during the first few weeks of observation was found in all dogs of which the early records were preserved (Experiments IV to VII). In addition to these four animals, three others were studied in which the pressure, at first high, fell within a few weeks. The pressures of these dogs are not included in this report, since when their blood pressure fell, the sounds became too faint to make accurate recording possible. The characteristic initial fall in pressure does not appear in the charts of the first three experiments because the early determinations were not recorded, as noted above. Petroff[6] has found a similar initial fall in pressure.

That the failure to take into account the characteristic initial fall in pressure is of importance, is evidenced by the work of Cash.[7] He draws conclusions as to the effect of various operative procedures on the blood pressure, from records in which this factor apparently has not been eliminated. In many instances he operated upon animals before the initial fall had ceased, as far as we can ascertain from the graphic records he presents.

The variation of blood pressure during the day was not investigated. To avoid the possible effect of food on the blood pressure, we made it a rule to take pressures at about the same time each day, before the meal hour. Petroff[6] has shown that there is usually a rise in pressure after the dog eats, which lasts several hours.

The particular advantage of this method is that it requires no preliminary operative procedure. The data which we have obtained compares very well with that of Petroff, who used a modified Van Leersum method involving an operative preparation of the carotid artery. The average pressures are similar to those of Allen[2] who used an indirect method slightly different from ours.

CONCLUSIONS

1. The auscultatory method described above is a satisfactory method of following the blood pressure in the unanesthetized dog over long periods of time.

2. The blood pressure of the dog tends to fall gradually during the first few weeks as he becomes accustomed to the procedure, until a relatively constant level is reached (Dogs. 13, 65, 67, 69).

3. The blood pressure of the normal dog tends to remain constant over long periods of time.

4. In the normal dog variations of systolic and diastolic pressure tend to parallel each other.

5. Inasmuch as blood pressures in dogs perhaps vary somewhat according to the age, size and breed of the animal, it is thought unwise to draw any conclusions from our data, as to the general average pressure of the animal. We may state, however, that the pressures we obtained agree quite closely with the results of other workers.

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A RAPID INEXPENSIVE METHOD FOR PREPARING PERMANENT TISSUE SECTIONS*

By Walter M. Simpson, M.S., M.D., F.A.C.P., and Helen H. Kreager, A.B., Dayton, Ohio

DIRECTORS of pathologic laboratories, particularly in hospitals not connected with teaching institutions, are constantly faced with the problem of standardizing laboratory procedures with two ultimate objectives: (1) to return an accurate report as soon as possible; (2) to economize on personnel and matériel. With these aims in view, we have adopted a method for the rapid preparation of permanent tissue sections which has survived a two-year trial period. By its use one technician has been able to make permanent sections of the tissues accruing from approximately 6,000 surgical cases a year, in addition to tissues derived from autopsies. These sections are ready for interpretation in most cases within twenty-four to twenty-eight hours after the specimen comes to the laboratory. All of the work in connection with the preparation of the sections is completed between 9 A.M. and 5 P.M. In fact, one tissue technician has had ample time each day to perform such additional special staining procedures as the demonstration of hemosiderin, fat, tubercle bacilli, and spirochetes.

Lane[1] recently published a description of a rapid method for the preparation of tissue sections. We have used that part of the Lane method which deals with dehydration and clearing. Some changes have been necessary in order to adapt this method to our time schedule. The following description gives the routine procedure which has proved most efficient and convenient and has resulted in the production of permanent sections of excellent staining quality and with a minimum of artefacts.

FIXATION

Blocks of tissue chosen for microtome sectioning are cut from the gross specimens before noon. All surgical specimens, except those in which bacteriologic studies are to be carried out, are placed in 10 per cent formalin solution in the operating room immediately after the operation. The tissue blocks may be selected from either a fixed or unfixed gross specimen. In all instances these pieces should not be more than 2 mm. thick. The cutting surface may vary, although 2.5 cm. is the maximum length and width unless special allowances are made when the tissues are imbedded. If the material for sectioning is selected from unfixed specimens it should be fixed rapidly by heating in a large test tube containing 10 per cent formalin solution. The best fixation, with minimum shrinkage, is obtained by heating just to the point at which air bubbles first appear and then allowing the tissue to remain in the hot formalin

*From the Diagnostic Laboratories of the Miami Valley Hospital, Dayton, Ohio.
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three to five minutes according to the size of the piece of tissue. The tissues should not be boiled. If speed is not essential the tissues may be fixed in 10 per cent formalin solution overnight. When the blocks are selected from the gross specimen all the pieces of tissue belonging to one case are placed in a four ounce wide-mouthed bottle half filled with waste alcohol. The proper identification number is then marked on the outside of this bottle with a wax pencil.

DEHYDRATION AND PARAFFIN INFILTRATION

The next step is to string all the blocks of tissue on a single length of thread. Number 60 white thread is sufficiently strong and a fine straight sewing needle is used. After the bottles are arranged in numerical order and their numbers written on a small pad of plain paper, one inch squares cut from heavy paper are numbered and arranged in sequence on a piece of filter paper leaving a space of about two inches between each square. Glazed Bristol board similar to that used for filing cards is suitable for this purpose; the numbers are written in pencil. The tissues are next removed from their bottles and placed on the filter paper immediately to the right of the piece of paper bearing the proper number. The pieces of tissue belonging to one specimen are counted, this figure set down on the pad beside the specimen number to which it belongs, and following it an estimate of the number of paraffin blocks this specimen will require when they are imbedded. Thus autopsy No. 104 has twenty pieces of tissue. Since eight of these are small and may be imbedded two in a block the number of blocks required is sixteen and the numbers appear on the pad as follows: A-104 - 20 - 16. We have found that these little records are useful for reference, and they are kept a month or so after the sections are finished. This being completed, the tissues are strung on the thread in order, the square of paper bearing the case number preceding the tissues belonging to it. By arranging them in this fashion the first square provides a good barrier and the following tissues are not in danger of slipping over the knotted end of the thread. Very friable tissue and small bits such as uterine curettings are folded in two layers of a small gauze square and threaded on in their proper place. This process of stringing the tissues takes only a few minutes and there is no danger of the tissues becoming too dry. The needle is then removed and the thread holding all the tissues for one day is dropped in the first of five one-quart jars. Common glass fruit jars with screw tops make excellent containers for the dehydrating and clearing reagents. In our laboratory these are placed in an out-of-way corner near the paraffin oven. Here the reagents are always lukewarm. Jar No. 1 contains 95 per cent alcohol in which the tissues remain for two hours. At the end of this time they are moved into Jar No. 2 which contains acetone. They remain in the acetone for fifteen minutes and for a similar length of time in jar No. 3 containing oil of cedar leaves. The fourth and fifth jars are filled with chloroform. The tissues remain in jar No. 4 (chloroform I) for fifteen minutes and in jar No. 5 (chloroform II) for forty-five minutes. At the end of this time they are ready for the paraffin bath in which they infiltrate overnight. It is evident that the time consumed in lifting the tissues from one jar to another is negligible. The total time used in prepara-

tion for imbedding is but three and one-half hours. Thus all that is required is to have the specimens in jar No. 1 by 1:30 P.M.; they are then ready for the oven at 5:00 P.M. With regard to the reagents, too much emphasis cannot be laid on the necessity of keeping them fresh. They are inexpensive and are easily procured. We have found it desirable to discard the used solutions and refill to the top of the jar once a week. The second chloroform solution may be transferred to the chloroform I jar and used for a second week but all others should be completely renewed. It is advisable to change the paraffin twice each week.

It will be observed that it is possible by this method to dispense entirely with absolute alcohol in the dehydration process.

IMBEDDING

After the overnight infiltration, imbedding and blocking is the first procedure in the morning. A well-chilled paraffin block is much more easily sectioned than a warm one. For this reason the tissues are blocked and put in the refrigerator as early as possible. Tin pans six inches long, two inches wide, and one-half inch deep are used for imbedding. Each of these will hold ten or twelve tissues. To prevent the hardening paraffin from sticking to the pan it is necessary to coat the inside surface of the pans with tincture of green soap just before they are to be used. The excess soap may be poured back into the stock bottle. Small pieces of paper of the same kind used in identifying the threaded tissues are kept on hand for use in imbedding. They should measure about one-fourth inch by one-half inch. Larger pieces are likely to curl before the paraffin is poured, thus making them difficult to remove later. These are numbered on both sides with pencil, one piece of paper being numbered for every tissue block to be imbedded. As they are numbered they are placed in the imbedding pan going from left to right across the width of the pan. After a tray of ice water is prepared, a Bunsen burner lighted and forceps laid out, the tissues are lifted from the paraffin bath to an enamel tray and a pint cup of melted new paraffin placed beside them on the bench on which the imbedding is to be done. Each group of tissues is slipped from the thread and placed with its number on the pan. Here a warming table could be used if desired. However, we have found that a very light flaming of each piece of tissue before it is imbedded is sufficient to melt the paraffin which has hardened on the tissues from contact with air. Of course, great care must be taken to avoid overheating of the tissues. The first imbedding pan is filled with paraffin from the cup and the tissues assigned to it are placed, one at a time after they are flamed, directly over the correctly numbered piece of paper on the bottom of the pan. It may be necessary to warm the paraffin in the cup over the flame just before it is poured into the pan; the paraffin must not be too hot because of the danger of burning the tissues. When the first pan is filled and a film of slightly congealed paraffin formed over the top by breathing over it, it is quickly submerged in the ice water and allowed to remain there until all the imbedding is finished. When the paraffin is thoroughly hardened the action of the water on the thin layer of green soap will permit the large block to float up from the tin pan.

The cold hardened paraffin containing the tissues is taken from the ice water, scored with a sharp scalpel between the tissues and the large slab broken into smaller pieces each containing the tissue to be mounted on one wooden block. These pieces are trimmed down on their four sides to within one-eighth to one-sixteenth of an inch of the tissue, the cutting surface leveled off and the paraffin square laid upon the wooden block having the proper number. The paraffin block is fixed to the wood by means of softening the under surface with a hot knife and pressing it down firmly on the wood.

Cubes of a soft light wood seven-eighths of an inch square are used for mounting the imbedded tissues. As these come to us from the carpenter, they have two surfaces sufficiently rough to hold the paraffin blocks securely, but since we boil these blocks in a strong solution of green soap, dry them and use them over again many times, these surfaces become smoothed out and it is necessary to pound several small holes in each of them in order to give the paraffin good anchorage. The laboratory case number is written with pencil on one of the four sides of the wooden block.

SECTIONING

A Spencer precision rotary microtome is used for routine work and all sections are cut within a range of four to six microns. The Warthin molasses plate method,[2] with slight modifications, is used for sectioning and staining. The necessary apparatus includes an iron ring stand with an adjustable ring, an iron ring tripod stand nine inches high, an enamel tray 8½ by 7½ inches, a dozen white enamel photo-trays 5½ by 7½ inches, a half dozen pieces of window glass measuring 7 by 5 inches, two or three heavy wire spring plate holders,* a paper hanger's wheel trimmer, small forceps with rounded and smooth tips, several camel's hair (No. 4 Russ sable) brushes and several right angle glass rods with the shorter of the two lengths measuring 5 inches to 5¼ inches. In preparation for sectioning, the large tray is placed on the tripod immediately to the left of the microtome. About 400 c.c. of a thin molasses solution is poured into the tray and heated with a low flame until it is lukewarm. The molasses solution is made from common New Orleans molasses (5 to 10 per cent) and distilled water. The thinner solution dries more quickly and is less sticky to handle. If the molasses is boiled vigorously when it is made up, a thymol crystal added and, after cooling, placed in the refrigerator and kept there, no difficulty will be encountered from the growth of yeasts or molds in the solution. The molasses in use is kept in a small bottle and is never poured back into the stock solution. One of the glass plates is thoroughly scrubbed, rinsed, and dried just before it is used. We find this is the best way to insure a clean surface for the sections, a detail which is highly important. After the wire holder is attached to the plate, the plate is put in the molasses in such a way that only about three inches of the top of the plate is above the solution. By resting the handle of the wire plate holder against the arm of the iron ring on the stand the plate may be adjusted so that as the exposed portion is covered with sections the plate may be drawn further out of the molasses by raising the ring and another row added. The pad of paper upon

*Obtainable from Eberbach and Company, Ann Arbor, Michigan.

which the tissue numbers and numbers of blocks have been written, a pencil, one of the Russ sable brushes, a section lifter or needle and a piece of dampened cloth are laid within easy reach of the technician before sectioning is started. After the paraffin blocks are trimmed down on the microtome, the brush, moistened and pointed in the molasses, is used to catch the first section and to hold the ribbon away from the knife as the sections come from the microtome. It is best to become accustomed to holding the brush with the left hand since the wheel of the microtome is on the right. After six or eight sections are cut the needle or section lifter is taken in the right hand and used to lift the knife-end of the ribbon away from the knife. The entire ribbon is then carried over to the molasses and laid down with the shiny or under surface of the sections on the molasses. The warmth of the molasses solution should flatten the sections without melting the paraffin. The pilot light of a Bunsen burner or a microburner will keep the molasses at this temperature while sectioning is being done. Unless many slides are to be made or serial sections mounted three or four sections are selected from several ribbons and the rest discarded. The sections are teased apart with the tip of the brush and the needle and the unused ones lifted out of the tray and wiped from the brush on the cloth. The sections to be used are guided on to the plate with the brush and arranged in rows from left to right. Care should be taken to moisten the exposed part of the plate with molasses with the brush before the sections are pushed up into position. Also a diagram showing the number of each block and its place on the plate should be made as the blocks are sectioned. This is necessary for reference when the sections are mounted. We find it possible to put sections from 15 to 20 blocks on one sheet in routine work. As each plate is filled with sections it is put in the paraffin oven to dry, a process which takes from fifteen to thirty minutes according to the thickness of the molasses solution. All the sectioning should be finished by noon each day which allows the plates to dry during the noon hour.

STAINING

About 400 c.c. of each of the following solutions are kept in bottles on the table where the staining is done. The exception is the celloidin which should be kept in a cool, dry place, preferably the refrigerator. All of these solutions may be used indefinitely. The stains should be filtered every third or fourth day and all of them will need to be replenished from the stock bottles about every second week. The alcohol solutions should be replaced about once a week. The following solutions are poured from the bottles into the photo-trays and staining is begun:

1. Tray containing Xylol No. 1. The dried plate with adherent sections remains in this tray until the paraffin is dissolved. This takes a very short time, since the paraffin has melted while the molasses solution dried in the oven.

2. Tray containing absolute alcohol, into which the plate is placed for a sufficient length of time to remove the xylol. The excess absolute alcohol is drained from the plate which is then held horizontally in the left hand while enough thin celloidin to cover the plate is poured over it. It should be poured

quickly so that it will run evenly over the entire plate before it has time to harden. After the plate is covered it is held in the same position for about fifteen seconds, then tilted and the excess celloidin allowed to run back into the wide-mouth glass-stoppered stock bottle. The edge of the sheet is evened off by running the paper hanger's trimmer around the four sides near the edge of the glass plate. This is important as the sheet will be inclined to catch and tear if the edges are rough or uneven. We use a thin solution of celloidin made of 8 grams of Parlodion and 200 c.c. each of absolute alcohol and ether.

3. Tray containing distilled water. By the time the edges of the sheet are trimmed the celloidin should be sufficiently dry to permit placing the plate in water. An over-dried sheet will be tough and will wrinkle while an under-dried one will cloud and become stiff. If the glass plate was thoroughly clean, as soon as the molasses dissolves in the water the sheet with the sections will float from the plate to the surface where it can easily be picked up with the bent glass rod. These rods must be perfectly clean.

4. Tray containing Mayer's hemalum solution. The sheet is lifted on the glass rod into this stain and opened out in it. Three to five minutes usually give a sufficiently deep stain.

5. Tray containing distilled water. The excess stain is washed off. The depth of the nuclear staining may be ascertained by floating the sheet onto a glass plate and examining it with a microscope.

6. Tray containing tap water. This is used as a mordant and the sheet should be allowed to remain until the sections have a deep blue black color. If the tap water available does not act as a mordant the addition of a few drops of a saturated solution of lithium carbonate will rectify this.

7. Tray containing 5 per cent eosin. The sheet is usually left in this solution for only five to ten seconds.

8. Tray containing distilled water. The excess eosin is washed off.

9. Tray containing 80 per cent alcohol. The sheet is moved back and forth on the glass rod several times.

10. Tray containing 95 per cent alcohol. The sheet should remain in this solution for several minutes. No harm is done if it remains in either of the alcohols while other sheets are started through the staining process.

11. Tray containing carbol xylol. This is made of 300 c.c. of xylol and 100 c.c. of melted phenol crystals. If the solution is too strong it will decolorize the sections and more xylol should be added. When the sheet is completely dehydrated it will sink to the bottom of the tray.

12. Tray containing xylol No. 2. The sheet is opened out and put in the proper position for mounting before the rod is removed. If the sheet becomes cloudy or sticks to the rod dehydration in carbol xylol was incomplete.

MOUNTING

Labeled and properly numbered slides are prepared before mounting. The sections to be used are selected, cut from the celloidin sheet with the paper hanger's trimmer and pulled up on the slide with the small tissue-section forceps. For routine work we find it a great saving of time and filing space to mount two or three sections from different blocks on the same slide, providing

they all belong to the same specimen. The slide with the sections is then blotted with filter paper, the glass around the celloidin wiped clean with gauze or a soft cloth, balsam dropped over the sections and the cover glass put on. If the staining and dehydration processes have been properly carried out the celloidin around the sections will be clear and colorless. Staining and mounting with this method requires about an hour. Thus the sections are ready for interpretation by the middle of the afternoon of the day following the arrival of the specimen.

SUMMARY

1. A method for the rapid preparation of permanent tissue sections is described. By this method it is possible to return a final report of the surgical pathology on the day following the operation.

2. The time and labor-saving qualities of the method make it possible for one technician to handle a large quantity of work.

3. The reagents and equipment are less expensive and more easily procured than those used in most other methods.

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A PERCUSSION BELL FOR STUDYING SOUND TRANSMISSION IN THE CHEST*

By Burgess Gordon, M.D., Philadelphia

IN THE use of auscultatory percussion and the so-called "coin test," the examiner requires not infrequently, the assistance of a colleague or the patient. It may be impossible to elicit sounds satisfactorily because of the difficulty in coordinating the positions of percussion and auscultation. The tuning fork is helpful since it may be operated by the examiner, but the prolonged vibrations obtained are frequently less desirable than a sound obtained by a single percussion impulse.

An instrument that has been helpful, especially in the study of pneumothorax, is constructed as follows: A bell 2.3 cm. in diameter and 2.4 cm. in depth is enclosed in a dome-shaped metal housing, 3.2 cm. in diameter and 3 cm. in depth. The bell is held in position by means of a hard rubber ring, a screw collar and plunger rod, which pass into the instrument through the dome of the housing and bell. A space 0.05 cm. in width separates the bell from the housing at all other points. A finger rest is screwed to the external

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The instrument is manufactured by George P. Pilling and Son Co., Philadelphia, Pa.

end of the plunger rod and to the end passing into the bell a cylinder with cam, spring and trip-hammer mechanism is attached. The cylinder is constructed downward and to the lowest point is screwed a hard rubber button which projects 1 cm. beyond the base of the bell. This serves as a sounding post and as a support for the trip-hammer mechanism. A hard rubber diaphragm with a central opening provides an outlet for the sounding post. The diaphragm is held in position by means of a screw collar at the lowest margin of the housing. An air vent 0.2 cm. in diameter is drilled into the dome of the housing. Two flanges are attached to the lower margin of the housing. These serve as finger grips.

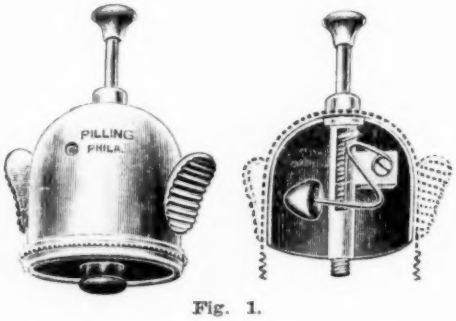

Fig. 1.

The operation of the instrument is as follows: The percussion bell is held between the thumb and middle finger and the index finger is used to operate the plunger. The instrument is placed against the chest wall in different areas and the plunger rod operated slowly as the examiner listens through a stethoscope. If a pneumothorax is present, the sounds heard will have a clear bell-like quality, due apparently to the resonance of a large cavity. Normal or solidified lung and simple effusions may yield merely a dull, muffled, distant or metallic note but in certain instances sharp variations in sound may be noted The study of these phenomena may be helpful in determining the nature of local processes, such as fibrous bands and changes in the bronchi.

TECHNIC FOR A CONSTANT SUPPLY OF GUINEA PIG HEART ANTIGEN*

By Perry J. Manheims, M.D., and Irene S. McGrath, B.A., New York, N. Y.

IT IS now recognized that guinea pig heart furnishes a more sensitive and stable antigen for the complement-fixation test than does beef heart. The following simple method of making both alcoholic and cholesterinized guinea pig heart antigen has been used by us for a number of years, and found very satisfactory. A glass bottle of approximately 150 c.c. capacity is cleaned, thoroughly dried, partially filled with 100 c.c. of 95 per cent ethyl alcohol, corked with a lead foil covered stopper, dated and placed in the refrigerator. As laboratory guinea pigs die from time to time in the process of cardiac bleeding for complement, the hearts are removed, freed from blood, blood vessels, and fatty tissue, quartered with scissors, and dropped into the bottle which is gently shaken for a moment at the addition of each heart. Only healthy guinea pigs are used and hearts of pigs which have been inoculated with any pathogenic organisms are discarded.

After about 20 hearts have been collected, the supernatant alcohol assumes a yellowish color. The presence of this color seems to be an indication that the antigen will be of proper strength. The finished antigen must contain from 30 to 35 hearts per 100 c.c. of alcohol, and the time for the completion of the alcoholic heart extraction varies according to the death rate of the guinea pigs bled for complement. There is a minimum period of extraction of six months from the time the first heart is placed in the bottle.

When the required number of hearts have been collected and the extraction period is complete, the yellowish alcoholic extract is poured off, filtered, and titrated; the hearts remaining in the bottle. Part of this antigen is cholesterinized, retitrated and used as cholesterinized antigen.

The bottle containing the hearts is then refilled with 75 c.c. of 95 per cent ethyl alcohol, redated, and with occasional shakings, allowed to extract in the refrigerator for from nine to twelve months longer. At the end of this period, the second alcoholic extraction is ready for titration. From these same hearts a third alcoholic extraction may be made by allowing a two-year period before pouring off the alcohol. The antigen from the second and third extractions is as strong as that from the first extraction. During this time other bottles of alcohol have been set up to receive fresh hearts so that there is always a supply of antigen in preparation, ready for titration and subsequent use.

Thus we have an extremely simple and economical method of preparing guinea pig heart antigen in constant supply with the expenditure of a minimum amount of labor and apparatus.

*From The Achelis Laboratory of The Lenox Hill Hospital.
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SUGGESTIONS FOR HANGING DROP PREPARATIONS*

By James B. McNaught, San Francisco, Calif.

TEMPORARY sealed drop preparations are often made by a very messy method of smearing petrolatum with a toothpick, match, brush or other article. The following neat time-saving procedures have been very valuable to us:

1. An ordinary lipped glass test tube is inverted and dipped into melted petrolatum then touched to the surface of a clean glass microscope slide (Fig. 1-*A*). Upon lifting the tube there is left on the slide a smooth ring of petrolatum ready for use. A cover slip containing a drop of material to be studied is inverted over the ring, slight pressure applied and the preparation is sealed. This method is extremely satisfactory for studying blood parasites, platelets and reticulocytes, for seeking parasites in stool emulsions and for classroom use where temporary sealed preparations of various kinds are needed.

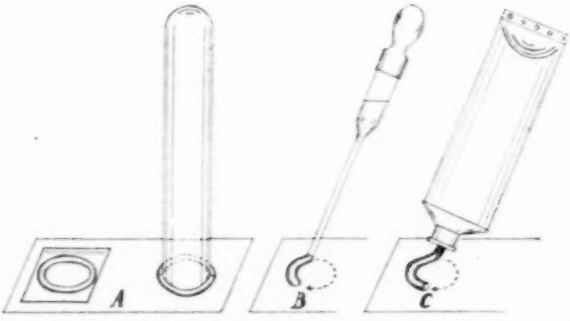

Fig. 1.—*A*, Method of forming a ring with an inverted glass test tube dipped in melted petrolatum and touched to the surface of a glass slide. *B*, Method of enscribing a ring on a glass slide with melted petrolatum expressed from a Wright pipette. *C*, Method of enscribing a ring with cold petrolatum jelly expressed from a tube with a punctured cap.

2. If a true hanging drop preparation is desired for suspending blood, bacterial suspensions, etc., from a cover slip, a thick smooth petrolatum ring may be rapidly made in the following manner: Melted petrolatum is drawn into a Wright pipette. Slight pressure on the nipple produces a continuous flow of melted petrolatum with which a circle of the desired size is rapidly enscribed on a glass slide (Fig. 1-*B*). The depth of the chamber is determined by the quantity of material expressed. If the petrolatum cools in the pipette, the whole may be passed through a flame until the contents are melted again.

3. A ring may be prepared from cold tubed petrolatum jelly. A hole is punched in the lead cap after removing the thin cork pad in the top of the cap. By squeezing the tube the petrolatum is forced out through the small hole in the cap and a circle enscribed on the glass slide (Fig. 1-*C*).

*From the Clinical Laboratory of Stanford University Hospital.
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SOME REFINEMENTS UPON THE COLORIMETRIC METHOD OF HASTINGS AND SENDROY FOR THE DETERMINATION OF THE P_H OF THE BLOOD*

By Janetta W. Schoonover, M.S., and Gladys E. Woodward, M.S.,
Philadelphia, Pa.

THE colorimetric method of Hastings and Sendroy[1] for the determination of the P_H of blood has been in constant use in this laboratory for the past eighteen months. During this time very satisfactory results have been obtained. This is no doubt due to following exactly the directions set forth in their procedure, accompanied, however, by further refinements. These, by reducing the possible experimental error, might eliminate some of the discrepancies which other workers may find between the colorimetric and electrometric methods.

Hastings and Sendroy, in their original paper, find that the colorimetric and electrometric methods agree with a maximum deviation of ± 0.04 P_H, and an average deviation of 0.003, when both determinations are carried out at 38° C. This observation has been confirmed recently by experiments carried out in this laboratory[2] in which the P_H of diluted plasma was determined by means of the glass electrode as well as colorimetrically, both determinations being carried out at 38° C. The close agreement (maximum deviation ± 0.03 P_H, average deviation 0.002) between these results indicates that the colorimetric method of Hastings and Sendroy measures the actual P_H of diluted plasma at that temperature. It is believed that this agreement is partly due to the careful control which we have practiced during our use of the colorimetric method. This control consists of strict adherence to every detail set forth by Hastings and Sendroy, with certain refinements which have resulted from our interpretation of their directions. Three of these, which in all probability promote the accuracy of the method, are discussed in the following paragraphs.

1. Arrangement of tubes selected for bicolor standard set

Since it is practically impossible to purchase test tubes of exactly the same internal diameter, it becomes necessary to use those whose diameters vary to a certain extent. Selection, by comparison of the 25 c.c. levels, of the 24 best from a recently purchased lot of 48, produced a set of tubes whose internal diameters showed a maximum variation of 0.7 mm. Therefore, it becomes imperative to employ the expedient of grading the tubes from one end of the range to the other, in order to avoid a derangement in color intensity which

*From the Cancer Research Laboratories, Graduate School of Medicine, University of Pennsylvania.
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may occur from a haphazard arrangement of the tubes. The seriousness of the derangement can be judged from the following example:

Suppose eight tubes, of which six have an internal diameter of 16.2 mm. and two of 15.5 mm., are chosen accidentally for the four pairs, P_H 7.30, 7.35, 7.40 and 7.45. The six tubes of the greater diameter compose the 7.30 and 7.45 pairs, the 7.35 alkaline tube and the 7.40 acid tube; the two narrow ones are used for the 7.35 acid and the 7.40 alkaline tubes. The diameter of each of the latter two is 15.5/16.2 or 95.7 per cent of any of the other six. The amount of color, then, per unit height, is 4.3 per cent less in these two than in the rest. The effect is a lowering of the intensity of the observed color, and, indeed, the exact P_H of the pairs of which they would be members can be calculated.

The 7.35 acid tube should contain, according to Hastings and Sendroy's data, 1.66 c.c. of 0.0075 per cent phenol red. However, its smaller diameter and consequent decreased intensity would cause this tube to appear as if it contained 1.66×0.957 or 1.59 c.c. This tube could not be distinguished by the eye from the 7.40 acid tube, which, according to Hastings and Sendroy's table, should contain 1.60 c.c. By similar reasoning, the decreased intensity across the 7.40 alkaline tube would make it appear to contain only as much dye as a 7.37 alkaline tube. If these so-called 7.35 and 7.40 pairs were compared in a color block, the only difference would lie in the alkaline tubes, which would vary but 0.02 P_H from each other. Thus, any readings between 7.30 and 7.45 would be inaccurate.

2. PERMANENCE OF THE STANDARDS

Hastings and Sendroy have shown that their standards kept for at least eight days without any significant change. In using this method for routine work, it is desirable to know the maximum length of time during which the standards remain accurate. This has been controlled for over a year in this laboratory, and it can be stated definitely that sets may be kept without any change for five weeks if they are not exposed to direct sunlight, and are kept in the dark when not in actual use.

The determination of the permanence of the standards was based upon the assumption that if two sets, one of which had been made up recently, agreed perfectly, both were correct. Therefore, sets were made up at definite intervals, one week, two weeks, and so on, so that each interval was one week longer than the preceding one. Each existing set was checked by the new set. Tubes in the oldest sets exhibited a fading which closely resembled a lowering of their P_H values, and which therefore could be recorded in that fashion.

There is a possibility of error in the preparation of any new set, but, if such an error occurs, it is evident when this set is compared to the preceding sets. In such event, another set is made up and the existence of the error confirmed.

A further check was made upon the accuracy of the standards by determining the P_H of phosphate buffer mixtures upon which hydrogen electrode

determinations had previously been made. The results agreed to within ±0.01 P_H.

Table I shows the variation which several of the sets have undergone with time.

TABLE I

FADING OF THE BICOLOR STANDARDS WITH TIME

SET NO.	DATE MADE	NO FADING	SLIGHT FADING	
		TIME	TIME	APPARENT FALL IN P_H
3	3/22/29	5 weeks	2 months	0 – 0.02
4	3/28/29	8 months	11 months	0 – 0.03
6	4/15/29	2½ months	2½ months	0
8	5/31/29	——	3 months	0 – 0.03
10	7/ 2/29	5 weeks	3 months	0 – 0.02
14	10/ 8/29	6 weeks	3 months	0 – 0.01
15	11/20/29	3 months	4 months	0 – 0.01
17	2/11/30	2 months	2 months	0
18	4/ 9/30	2 months	3 months	0 – 0.01

It will be seen that all of the sets keep perfectly for at least five weeks, while most of them are good for two months. Set No. 4 showed no change in eight months, and was still good enough for use after eleven months. Only one tube out of the 24 showed the maximum variation given in Table I. This set, however, was not in general use, and therefore was less exposed to the light than other sets. This confirms our opinion that the principal change in a color tube is one of fading brought about by light. One set, which was kept in diffuse light, showed the greatest change of all. In three months some of the tubes had faded to the extent of 0.09 P_H. Therefore, unless this error is avoided, a P_H reading with such a set would be much more alkaline than the correct one. It is suggested, for this reason, that two sets, at least, be kept on hand, and renewed alternately every five weeks, so that the new standards may be checked with a set which has not had time to fade.

3. ADJUSTMENT OF THE SALINE INDICATOR

Hastings and Sendroy recommend that the saline indicator, already in the P_H tube and covered with oil, be adjusted to a P_H of 7.40 with 0.01 N. sodium hydroxide, delivered through a capillary pipette below the oil. This procedure may be altered for rapid work by the adjustment of the total amount of indicator required for the day.

There may be times when the stock solution of indicator has fallen to so low a P_H that the amount of sodium hydroxide required may have an appreciable dilution effect upon the indicator color. The resultant lesser intensity may easily be mistaken for a lower P_H when the sample tube is compared with the standards.

In order to avoid this dilution error, adjustment of the saline indicator is best accomplished by adding to the stock solution dropwise, and with slight agitation, a solution of 0.01 N. sodium hydroxide, containing, however, phenol red and sodium chloride in the same concentrations as those of the solution whose P_H is being regulated. This procedure keeps the indicator and salt concentrations constant, and may make a series of determinations slightly more rapid.

SUMMARY

1. The colorimetric method of Hastings and Sendroy for the determination of the P_H of blood has been used with satisfactory results in this laboratory.

2. The following refinements have been developed:

a. The tubes used for the bicolor standards have been graded according to size.

b. The period of reliability of the standard sets has been found to be at least five weeks.

c. The saline indicator solution is adjusted by means of 0.01 N. sodium hydroxide containing the proper concentrations of phenol red and sodium chloride.

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A HEART SOUND AMPLIFIER*

By Russell A. Waud, M.D., Ph.D., London, Canada

VARIOUS electrical circuits employing the thermoionic valve have been described for the amplification of heart sounds and small currents of physiologic origin. H. B. Williams[1] recorded heart sounds by this method in March, 1921, and Gen. G. O. Squier[2] in June, 1921, described a method of amplifying and broadcasting heart sounds; since this pioneer work various circuits have been described.

In institutions where the expense of a factory-made instrument is prohibitive and the services of one at all familiar with the principles of radio are available, the data described here may prove valuable.

The circuit is essentially an ordinary three tube transformer coupled amplifier with a one tube preamplifier to bring the energy generated by the microphone up to the value of that produced by an ordinary phonograph pick-up.

THE MICROPHONE

It is now recognized that the carbon type of microphone is unsatisfactory for reproducing heart sounds; the frying noises which are always present, when amplified to the degree required in heart sound work are nearly as loud as the sounds produced by the heart itself. The condenser type is not only quite expensive but also the sensitivity is much less than the other

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types. The microphone of choice is therefore the electromagnetic, the principle of which is essentially that of the ordinary telephone receiver. When used as a receiver a current passed through the coils of the magnet causes the diaphragm to vibrate, while when a microphone is required, the diaphragm is set in vibration by sound waves and this sets up currents in the coils. In fact a very good microphone for heart sounds can be made by slightly modifying a high resistance radio head phone. However as this type requires careful adjustment and is frequently damaged by rough handling, the Utah piano unit, a more rigid type, has been adopted in this work. The metal strip used to fasten the unit to the piano sound board is replaced by a circular piece of brass 3.5 cm. in diameter and about 0.8 cm. in thickness. When in use this piece of brass is placed against the patient's chest.

Because of the high sensitivity of the amplifier it is necessary that the wires leading from the microphone to the amplifier be shielded. Ordinary copper shielding serves very well for this purpose. The copper should be connected to the ground of the amplifier.

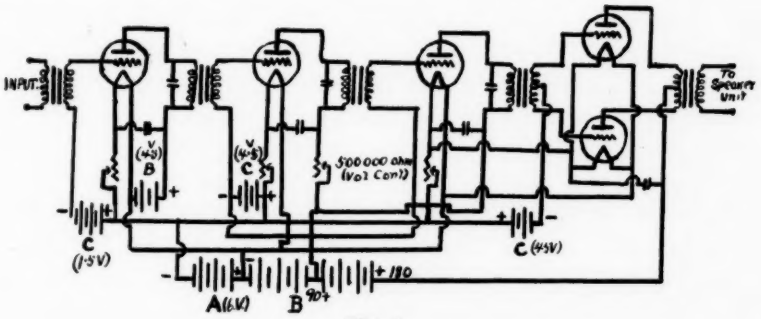

Fig. 1.

THE AMPLIFIER

A diagram of the amplifier and preamplifier is shown in Fig. 1. As stated above with a few exceptions, the circuit is essentially that of an ordinary transformer coupled amplifier. To heat the filaments a six volt wet battery is used. Because of frequent "line noises" heating the filaments with alternating current is not satisfactory. The high voltage current is derived from ordinary "B" batteries as any type of "B" eliminator is unsatisfactory, it being impossible to eliminate the background hum. It will be seen that the energy generated by the microphone (transmitter) first passes into the primary of an audio frequency transformer and the secondary of the transformer is connected to the grid of the 201A tube of the preamplifier. The preamplifier is essentially a part of the main amplifier with the exception that it has a separate 45 volt "B" battery. The main amplifier consists of two stages of single transformer coupling and a final stage in which the current is divided between two tubes, this allows a large amount of current to pass without distortion. In order that a small portion of the energy in the last tubes does not pass back along the coupling wires to an earlier tube, be magnified and appear as a whistle or howl, condensers of two microfarads capacity are connected between the positive and the negative terminals of the

"B" batteries. In the first two stages of the main amplifier 201A tubes supplied with 90 volts of "B" battery are used, while in the final stage two 171A power tubes with 180 volts is most satisfactory. Ferranti transformers were used throughout.

FILTER SYSTEM

As it is sometimes desirable to suppress sounds of high frequencies and at the same time preserve those of low pitch; four fixed condensers varying in capacity from 0.25 to 2 microfarads are placed across the secondary of the last transformer. By means of a multiple switch any one of these can be brought into use. They are also very effective in eliminating noises originating in the room which are as a rule high pitched and are also confusing.

OUTPUT

For the changing of the electrical variations into sound waves a "Baldwin Concert Grand" unit was used, the horn however was replaced by a Bowles multiple stethoscope allowing twelve individuals to listen at one time, the chest piece should be removed from the stethoscope and the rubber tubing connected directly into the unit. In using the instrument the ear pieces should not be placed directly over the auditory meatus but should be allowed to hang loosely in the pinna; if this precaution is carried out, the sounds heard through the amplifier are the same in all respects as those heard in the ordinary way with the exception that they are very much louder.

In order to make the instrument portable it can be housed in a box and the box mounted on wheels similar to those used on a dinner wagon. The controls can be placed on the top and the amplifier and batteries below. A trickle charger connected with the wet battery will keep it in good condition.

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THE USE OF SOLID CARBON DIOXIDE (DRY-ICE) IN THE FREEZING OF TISSUES FOR MICROTOME SECTIONING*

By F. Lowell Dunn, M.D., Omaha, Neb.

THE usual method of freezing tissues for microtome sectioning is by means of carbon dioxide delivered from tanks containing the liquid under about 1000 pounds pressure. The sudden evaporation of carbon dioxide through a small orifice results in cooling and formation of solid carbon dioxide which freezes the tissue.

Solid carbon dioxide can be readily obtained; commercially it is sold under the trade name of "Dry-Ice." The freezing of tissues can be carried out more efficiently by its use, dispensing with the tank and freezing attachment. This change is economical because the freezing attachments furnished with freezing microtomes are in general quite inefficient compared to the devices in use industrially for the manufacture of "Dry-Ice." The interesting development of the "Dry-Ice" industry and its many uses has recently been described by Killefer.†

The adaptation of the standard freezing microtome to the use of "Dry-Ice," or solid carbon dioxide is a simple matter. This is partly because the subliming temperature of solid carbon dioxide is only about –110° F. The essential changes are (1) a metallic pathway to conduct the cold from the "Dry-Ice" to the tissue, and (2) suitable insulation so that cold is not lost to the microtome frame. These requirements can of course be met in many ways. Fig. 1 illustrates a very simple but satisfactory method. *A* is a fiber insulating rod which is clamped in the jaws of the microtome specimen holder. *B* is a brass cap on which the specimen is placed and *C* is a piece of flat brass. The block of "Dry-Ice" is held with a towel or gloves against *C*. Although "Dry-Ice" can produce serious frost-bites, Killefer states that the use of even thin gloves is sufficient protection. Freezing is prompt, complete, and, due to the larger heat capacity of the attachment and the coldness of the "Dry-Ice," the freezing is of longer duration. The above attachment was used on a Bausch and Lomb Optical Company Automatic Laboratory Microtome. It is unnecessary to describe the conversion of the ordinary freezing attachment to using "Dry-Ice." The use of clamps, cups, or other devices for holding the "Dry-Ice" in place of *C*, Fig. 1, is probably unnecessary since the freezing time is so short.

"Dry-Ice" is being produced in 18 plants in the United States and is widely distributed. Its use in the transportation of ice cream has resulted

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†Killefer, D. H.: The Growing Industry—Dry-Ice, Indust. & Engin. Chem. 22: 1087-91, 1930.

in definite economies so that it can usually be obtained from ice cream manufacturers for a few cents a pound. In our experience one pound will suffice to freeze 25 to 50 small pieces of tissue.

The preservation of "Dry-Ice" is discussed by Killefer.† Because of its direct evaporation there is no moisture. This increases the efficiency of various insulating media and permits the use of more efficient materials than is possible when ice is the refrigerant. Killefer states that Kapok fiber, balsa wood, and several of the wood-fiber boards are proving highly success-

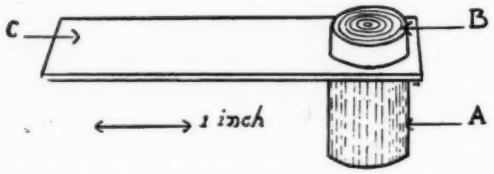

Fig. 1.—*A*. Heat insulating material such as hard rubber or bakelite. *B*. Platform for tissue. *C*. Conducting plate against which "Dry-Ice" is held.

ful. Large cakes of "Dry-Ice" will of course keep better than smaller pieces, and for this reason we have used the block of solid carbon dioxide for freezing tissues without breaking it up. Thermos bottles are fairly satisfactory for keeping "Dry-Ice."

SUMMARY

Solid carbon dioxide purchased in blocks as "Dry-Ice" furnishes a cheaper and more efficient method of freezing tissues for sections than the use of carbon dioxide in tanks.

A CLINICAL METHOD FOR THE ESTIMATION OF URINE SUGAR*

By Jerome E. Cook, M.D., and Alexander Steiner,† M.S., St. Louis, Mo.

A STUDY of Trommer's qualitative test for urine sugar led Salkowski in 1872[1] to the observation that in the first phase of the procedure copper hydroxide combines with glucose to form a well defined compound which is precipitated. In the second phase, this dissolves and subsequently undergoes decomposition with the formation of cuprous oxide. Fileti[2] and later Yoshimoto[3] corroborated Salkowski's findings and showed beyond doubt that under given conditions copper and glucose combine in definite proportions.

One of us has been utilizing this fact for many years in his routine clinical uranalysis for the semiquantitative determination of urine sugar. Trommer's test, which with the advent of newer copper reagents has rather generally come to be considered obsolete, is performed as follows: The sugar solution (urine) is rendered strongly alkaline by the addition of alkali hydroxide, then copper sulphate solution is added dropwise as long as the initially formed precipitate continues to go into solution. When the first permanent turbidity occurs in the mixture (which exhibits the blue color of alkaline copper tartrate solutions) the first phase of the test is ended. The mixture is then heated to the boiling point and the glucose-copper hydroxide compound decomposes with the formation of cuprous oxide.

Salkowski's observations were concerned only with the precipitated glucose-copper hydroxide compound. We have not at this time gone further into the study of this compound. Our interest has been centered in the blue alkaline copper-glucose solution with the object of ascertaining whether there are conditions under which the copper is held in solution in amounts proportional to the amount of glucose present. We have found that such conditions do exist and that a most important factor affecting this proportionality is the concentration of the alkali. A 5 per cent concentration of sodium hydroxide was found to be optimum for the purpose. This is obtained by the addition to the urine of an equal volume of 10 per cent sodium hydroxide. A proper amount of 10 per cent copper sulphate is added to this mixture. Under these conditions a quantitative ratio of copper solubility exists within the range of sugar concentrations from 0.5 per cent to 3.0 per cent. If less than 0.5 per cent of glucose is present, a few drops of the 10 per cent copper sulphate solution will give rise to a permanent turbidity, and it may be difficult to judge whether any of the copper hydroxide has gone into solution. Heating the mixture, i.e., completing the Trommer test in its original qualitative form, will decide the question. If on the other hand, the copper sulphate consumption is in excess of 3.0 (indicating a glucose content over 3 per cent), the urine must be diluted for quantitative determination.

*From the Medical Service and the Laboratory of the Jewish Hospital of St. Louis. Received for publication, November 8, 1930.
†Working with a grant from the Selma Michael Research Fund.

The simplest way of carrying out the test in a clinical laboratory is as follows:

Into a clean 16 mm. test tube, graduated to 5, 10, 11, 12 and 13 c.c., introduce clear urine to the 5 c.c. mark, add 10 per cent sodium hydroxide to the 10 c.c. mark and mix well. Immediately add to the mixture of a 10 per cent solution of copper sulphate, 2 or 3 drops at a time, shaking vigorously after each addition. The copper hydroxide precipitate initially formed will in the presence of glucose readily go into solution on shaking, imparting a blue color to the urine-sodium hydroxide mixture. This color deepens as the addition of copper continues, but the solution remains clear or only very slightly turbid until a saturation point is reached when the addition of another drop or two of the copper increases very sharply the turbidity. This is taken as the end point and the amount of copper sulphate solution used can be read off directly on the graduations of the test tube above the 10 c.c. mark, the fractions being estimated. The volume of added copper sulphate solution in cubic centimeters is identical with the percentage concentration of glucose; thus, for instance, if the copper sulphate used is 2.7 c.c. the glucose content of the urine is 2.7 per cent. If as the test is done, the fluid level goes beyond the 13 c.c. mark, i.e., if the end point is not reached by the addition of 3 c.c. of copper sulphate, a fresh portion of urine is taken, diluted with an equal volume of distilled water and the test repeated with 5 c.c. of this diluted urine. The final reading is of course multiplied by 2 to correct for the dilution.

In performing the test it will be found advantageous to drop the copper sulphate into the test tube held somewhat at a slant, so that the drops hit the wall of the tube a few cm. above the fluid level. This will facilitate the dispersion and solution of the precipitate at first formed. It goes without saying that the copper and sodium hydroxide reagents must be free from turbidity. Because of the instability of the glucose-copper compound it is necessary that the temperature of the urine should not be above normal room temperature or else a decomposition with the formation of cuprous oxide may set in before completion of the procedure, especially if the concentration of the sugar is high. For the same reason the entire procedure should be carried through with reasonable celerity.

The test has the same disadvantages encountered in all other copper tests but these can usually be easily obviated. Highly concentrated, dark urines have some copper binding ability due to substances other than glucose which will interfere with the accuracy of the reading. Albumin in any considerable amount interferes with accuracy, and should first be removed. Certain drugs, sugars other than glucose (lactose and arabinose) and bile may likewise render copper tests for glucose inaccurate.

To illustrate the degree to which accuracy can be approached by our method, comparative determinations of sugar in urine were performed by three methods: (1) The Shaffer-Hartman method (Somogyi's modification)[4]; (2) Somogyi's fermentation[5]; and (3) the method above described. The results are given in Table I.

TABLE I

NO.	SHAFFER-HARTMAN METHOD	SOMOGYI'S METHOD	NEW METHOD
	PER CENT GLUCOSE		
1	1.70	1.6	1.7
2	5.10	4.7	4.7
3		0.8	0.8
4	2.65	2.2	1.8
5	1.28	1.2	1.3
6	3.80	3.5	3.0
7	1.96	1.8	1.5
8	1.87	1.7	1.8
9	3.28	3.0	3.5
10	0.89	0.8	0.7

The figures in Table I show that our method yields results which are in fair agreement with those obtained by the two other procedures. It may be remarked that the Shaffer-Hartman method, like all copper reduction methods, yields results that are from 0.1 per cent to 0.3 per cent too high since they include reducing substances other than sugar, while Somogyi's method gives true sugar values. Our results seem to agree better with the latter.

On this basis we believe that with a little practice the glucose content of the ordinary diabetic urine can be estimated by the method described with an accuracy which would seem to answer every clinical requirement for a quantitative test. At the same time the procedure is sufficiently simple and rapid to warrant its use in place of the usual routine qualitative tests.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SYPHILIS: Further Studies on the Precipitation Test, Weiss, E. J. Infect. Dis. 47: 355, 1930.

The author has devised an antigen which has the following advantages over Kahn's antigens: The technic of preparation is simpler; the specific zone is considerably wider; it does not require additional sensitizing or correcting.

Dry beef heart is extracted with ether in the ratio of 1:13 for forty minutes in the ice box. The extract is then discarded and the tissue dried. To the dried tissue 95 per cent alcohol is added in the ratio of 1:5. The alcoholic extraction is carried out at 37° C. for three days. This extract is then filtered and left overnight at room temperature. The extract is refiltered and 0.6 per cent cholesterol is added.

DIPHTHERIA IMMUNIZATION: Percutaneous Method of Loewenstein, Abt, A. F., and Feingold, B. F. Am. J. Dis. Child. 41: 8, 1931.

The method is as follows:

Either the back, the chest and abdomen or the arms can be used as the site for the rub. The choice in each case depends on the size of the child. In smaller children the arms do not offer a sufficiently large absorbing surface, so that either the back or the chest and the abdomen should be used. The skin is prepared with ether to remove the natural body oils and scales. This is followed by the application of alcohol, which is allowed to dry. The contents of a single tube containing the preparation are applied and rubbed in, either with the finger tips or a small wooden spatula. This takes from about two to three minutes. The ointment dries shortly, leaving a yellow coating resembling in appearance dried glue or shellac. The surface is not washed for twenty-four hours.

Twenty-six, or 55.3 per cent of 47 children who were given three inunctions of Professor Loewenstein's ointment for active immunization against diphtheria gave negative reactions to the Schick test within six weeks.

No local or general reactions were noted.

LIPOIDS: In Nephrosis and Chronic Nephritis With Edema, Lichtenstein, L., and Epstein, E. Z. Arch. Int. Med. 47: 122, 1931.

Blood chemistry determinations have been made before, at and one hour after death, on 33 nondiabetic patients dying in the hospital.

Among these 33 unselected cases, 12 cases, or 36 per cent, showed low terminal blood or plasma sugars, ranging from 28 to 75 mg. per 100 c.c. at the moment of death.

In a number of cases it was demonstrated that this pronounced and suggestive hypoglycemia existed before death as well as at death.

Only a slight decrease was noted in the blood sugar during the hour immediately following death, although there were two noteworthy exceptions in which the sugar of the heart blood decreased from 154 to 50 and from 166 to 32 mg. during the first postmortem hour.

The blood urea frequently increased rapidly just prior to death, but little change took place after death.

Frequently there was a terminal rise in amino-acid nitrogen, which continued to increase somewhat after death. The carbon-dioxide combining power of the plasma decreased somewhat shortly after death, but the chlorides remained unchanged.

PNEUMONIA: Prognostic Significance of Leucocyte Count in Pneumonia in Children, Meyer, H. F. Am. J. Med. Sc. 181: 707, 1931.

In a series of 100 cases of pneumonia in infants and children, it was found that the mortality was inversely proportional to the leucocyte count, except that in children having over 50,000 cells per c.mm.

In this series of pneumonias, regardless of type, there is a relation between the lack of response on the part of the body in the production of leucocytes and a high mortality.

Evidence is presented which indicates that the total leucocyte count offers a valuable method of approximating the reaction of the host to the infection, and hence may be utilized as a prognostic aid.

Suppurative complications and extension of the pneumonia may be anticipated even in the presence of the primary pneumonia by the sudden increase of the leucocyte count.

CHOLESTEROL: Partition of the Blood Plasma in Parenchymatous Diseases of the Liver, Epstein, E. Z. Arch. Int. Med. 47: 82, 1931.

An added insight into the complex problem of liver function seems to be gleaned from the determination of the total and cholesterol ester, a simple method that requires only from 1 to 2 c.c. of blood plasma, and that can be repeated throughout the course of the disease.

Ten cases of acute diffuse parenchymatous damage of the liver have been studied. The cholesterol ester values in the blood plasma were found to be diminished or entirely absent, corresponding with the severity of the diseased process. With the improvement in the condition the ester values rose to their absolute and relative normal proportions. This progress as revealed by the ester values paralleled in large measure the general condition of the patient and the other liver function tests.

In four cases of atrophic cirrhosis of the liver, with its slow evolution, slight damage and ample regeneration, the cholesterol partition was normal.

The ester values seem to offer some means of diagnosing parenchymatous damage of the liver and, when repeated during the course of the disease, some prognostic significance.

BLOOD PRESSURE: In Infancy and Childhood, Abt, A. F., and Feingold, B. F. Am. J. Dis. Child. 40: 1285, 1930.

The authors present a thorough and comprehensive review which they summarize as follows:

1. The Riva-Rocci mercury manometer with a 9 cm. cuff is the instrument best adapted for the determination of blood pressure in children.

2. There is no agreement in the literature as to blood pressure in normal infants and children.

3. There are two great groups of variables: a more constant and a less constant. The more constant group includes age, height, body weight, sex, circumference of the arm, race, heredity and climate. The less constant group of variables includes position of the body, pulse, temperature of the body, atmospheric temperature, relation to meals, motions, sleep, fatigue and exercise.

4. It has been pointed out that astonishingly high blood pressures, from 150 to 160 mm. of mercury systolic, may be found in normal adolescent children. These pressures fall without any treatment and without in any way impairing future health.

5. Sudden changes in blood pressure accompany sudden losses and gains in weight occurring in the infant during nutritional disturbances.

6. Blood pressure in children with chronic heart disease may be not only normal, but elevated.

7. Notwithstanding a diversity of opinion, we believe that determination of blood pressure in pneumonia is a valuable index to the integrity of the circulatory system.

8. In children with pulmonary tuberculosis, the blood pressure is generally lowered.

9. It is generally agreed that blood pressure is lowered in the infectious diseases.

Toxemia is a more important factor than pyrexia in diminishing the blood pressure in the infectious diseases. A decreased pulse pressure due to vasomotor failure indicates an unfavorable prognosis in infectious diseases.

10. Hypotension occurs as the result of the toxemia in diphtheria.

11. There is a general disagreement in the blood pressure observations reported for scarlet fever during the acute state. The frequency of the occurrence of postscarlatinal nephritis with hypertension varies with different studies. Until conclusive evidence is offered to prove the relationship between scarlet fever and chronic interstitial nephritis in older children and adults, the importance of this disease as an etiologic factor in chronic hypertension cannot be estimated.

12. The blood pressure may be regarded as an index of prognosis in acute nephritis. A regular pulse with increased blood pressure is favorable, while hypotension and cardiac arrhythmia are bad prognostic signs. An impending uremia complicating an acute nephritis is always preceded by a steady increase in arterial tension.

13. Chronic nephritis may or may not be associated with increased arterial tension. Hypertension is always found in: (a) chronic diffuse nephritis of the primary renal type with a preceding history of an acute glomerular nephritis or a prolonged sepsis and in (b) the primary arteriosclerotic type of chronic diffuse nephritis with secondary contraction of the kidney. Chronic diffuse nephritis of the primary renal type without a history of acute nephritis or preceding sepsis is characterized in the majority of cases by a normal blood pressure which is raised, is rarely above 150 mm.

14. Comparatively few cases of true essential or malignant hypertension in children have been reported in the literature up to the present time. It is doubtful whether these cases can be separated from chronic nephritis of the arteriosclerotic type (type b).

15. With any rise in intracranial pressure there is a corresponding rise in blood pressure. Intracranial hemorrhage produces a rise in blood pressure. In fractures of the skull a steady rise in blood pressure is evidence of a progressive hemorrhage. Tumors of the brain and other diseases producing intracranial pressure are accompanied by hypertension.

In epilepsy blood pressure is normal during the interval between seizures. Prior to and during an attack, blood pressure is elevated.

16. In hyperthyroidism there is a high pulse pressure. In cretinism, Addison's disease and Fröhlich's syndrome there is a hypotension.

In diabetes, blood pressure is normal or slightly below normal. Insulin produces a fall in pressure.

17. A variable hypertension has been reported for infants with exudative diathesis, presumably due to constitutional anomalies in the circulatory dynamics.

18. Although there is a dearth of information concerning the effect of drugs on blood pressure in children, the authors infer that their action is similar to that reported in adults.

TISSUE: Rapid Method for, Geschickter, C. F., Walker, E. P., Hjort, A. M., Moulton, C. H. Stain Tech. 6: 3, 1931.

1. A buffer solution or prestaining bath, of the following composition, is used to collect the sections as they come from the microtome:

Potassium acid phosphate	6.75	gm.
Normal sodium hydroxide	30	c.c.
Distilled water	570	c.c.
Glycerin	200	c.c.
95 per cent alcohol	200	c.c.

The ingredients should be mixed in the order given.

2. The sections are then passed into the following solution for twenty to thirty seconds: thionin eosinate 0.75 gm., barium eosinate 0.25 gm., azure A 0.25 gm., dissolved in 100 c.c. of a mixture of 4 parts ethylene glycol and 1 part ethyl alcohol (95 per cent) plus 0.2 per cent glacial acetic acid. Other stain combinations, using the same solvents, are under investigation.

3. The excess of stain is then washed off in two successive changes of 95 per cent alcohol containing 20 c.c. of glycerin to each 80 c.c. of alcohol. The tissue is left in the first bath about ten seconds and washed back and forth and is then transferred to the second bath for about three seconds. If the sections are too blue, wash longer in the second bath or add two or three drops of 5 per cent glacial acetic acid to the first bath.

4. The section is next dehydrated by floating for ten or fifteen seconds in a solution of diethylene glycol monobutyl ether.

5. The section is now cleared in n-butyl phthalate by floating in this solution twenty seconds.

6. The section is now floated on a glass slide, blotted dry by means of photographic lintless blotting paper, and a cover slip upon which a drop or two of gum damar has been placed is mounted over it.

STAIN: Elective Staining of Basophilic Granules in Mastocytes, Byard, E. Bull. d' Histol. Appl. **7**: 264, 1930.

This is a method for the elective staining of basophilic granules in mastocytes. Fix in either alcohol, formalin-saline, alcohol-formalin, bichromate-formol. Stain deparaffined sections in 1 per cent aqueous acid fuchsin, thirty seconds. Rinse rapidly in water. Treat with a 0.8 per cent aqueous solution of bromine, four to five minutes, until violet, in a closed container (the exact concentration is very important). Wash in water. Differentiate in 95 per cent alcohol or acid alcohol (1 per cent HCl), one to three minutes, until light mauve. The fuchsin precipitate should be dissolved out of all the preparation except the granules. Complete differentiation and dehydration with absolute alcohol, exercising care not to destain mastocytes. Clear in xylol; mount in balsam. The granules are carmin red while the nuclei and cytoplasm are colorless.

Nuclei may be stained after the granules if desired. In this case rinse in water after differentiation of the granules. Mordant in 4 per cent iron-alum four minutes at 50° C. or forty to fifty minutes at room temperature. Stain with Regaud's hematoxylin for the same time at the same temperature. Differentiate in 1 per cent iron-alum and proceed as usual, timing carefully the alcohols. Nuclei are black, granules red.

ANEMIA: Pernicious, Neutral Red Test in, Cohen, S. J., Matzner, M. J., and Gray, I. Arch. Int. Med. **46**: 979, 1930.

A Rehfuss tube is introduced into the "fasting stomach" and the stomach is washed with water until the contents return water clear. Two cubic centimeters of a 2 per cent solution of neutral red (40 mg.) is injected intramuscularly into the gluteal region. The stomach is then aspirated every fifteen minutes for two hours. Each specimen is kept in a separate test tube. In cases in which there is very little or no gastric secretion, a quantity of water, 15 or 20 c.c., is introduced into the stomach through the tube and aspirated at the end of the fifteen-minute periods.

The gastric contents are then treated in the following manner: To 15 or 20 c.c. of the contents of the stomach, approximately 2 gm. of tribasic lead acetate is added, and the tube shaken. If the contents seem to contain a large quantity of bile, about 1 gm. of purified animal charcoal is also added. This is filtered through filter paper, and within a few minutes sufficient filtrate is obtained to fill a small test tube. When smaller quantities of the contents of the stomach are used, proportionate amounts of the lead acetate and charcoal are employed. A few drops of glacial acetic acid are added to the filtrate while it is viewed against a pure white background (such as white paper) and while a control tube with plain water is held next to it. When neutral red is present the solution will begin to assume a pinkish color within a few seconds. The depth of the color is proportional to the concentration of the dye.

After applying the test to three cases the authors conclude that the value of the test for neutral red is always questionable because the presence of the dye in the stomach may be accounted for by regurgitation of the duodenal content.

The presence of neutral red in the gastric extractions does not in itself exclude the diagnosis of pernicious anemia.

URINE: New Clinical Method for Estimation of Protein, Kerridge, P. M. T. Lancet **220:** 5601, 21, 1931.

Reagents: (1) A. 1:25 solution of Higgins' "Eternal Ink."
(2) B. Trichloracetic acid 100 per cent.

Standards: Commercial horse-serum the protein N of which is determined by micro-Kjeldahl. Amounts containing 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, and 4.0 mg. protein N are placed in 3 × ⅜ in. tubes and the volume made up to 1 cm. with distilled water. To each add 0.1 cm. of Sol. A. & B., shake, and cork. Standards correspond to 0.025, 0.050, 0.075, 0.1, 0.15, 0.2, 0.3 and 0.4 protein N mg. per cent.

Test: Place 1 c.c. of urine or cerebrospinal fluid in a 3 × ⅜ in. tube. Add 0.1 c.c. of A., shake and add 0.1 c.c. of B. Shake. Compare gray shade resulting with those of standards and read.

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EDITORIALS

Sickle Cell Anemia

VARIOUS abnormalities in the erythrocytes of man have been observed since the earliest microscopic studies of blood. Today variations in size, shape and content of the cells are recognized both in health and disease, although in health the morphology and chemistry of erythrocytes is decidedly uniform.

French workers as far back as Hayem described semilunar erythrocytes, and later they rather definitely related them to malarial infections. It is unlikely that these references pertain to a condition known as sickle cell anemia, which is almost wholly dealt with in American literature.

It is not unlikely that Dresbach, in 1904, first observed sickle cells, since his patient was a mulatto, although he did not establish the clinical entity. For this achievement credit must go to Herrick who, in 1910, described the condition and associated it with severe anemia in a negro who presented a complicated picture of yaws, hookworm, a cardiac lesion, general adenopathy, icterus and anemia. Many of the erythrocytes were distorted into sickle-shaped and crescent-shaped forms.

Although many papers have appeared since that of Herrick, it was Mason, in 1922, who labelled the entity sickle cell anemia, and this name has remained ever since. The more euphonious name of "sicklemia" is used to designate the tendency to form sickle cells.

After 1910, numerous observations were made on the disease, prominent among which were those of Sydenstricker. The malady was thought to be confined to the negro race, but in 1927 Lawrence found sickle cells in the blood of a white woman, her brother, a sister, and a niece. He also found such cells in three of 102 white persons, although Sydenstricker did not observe them once in examinations of 1,000 white persons. The report of Lawrence of course strengthens the record of Costana who in 1925 reported gigantocytes of a semilunar form in a white child and enhances Stewart's report of sickle cells in a white child. Cooley and Lee more recently reported the condition in two members of a Greek family. In spite of these exceptions, evidence supports the contention that the condition thus far observed is almost wholly encountered in blood of negroes and may be found in from 5.5 to 7.5 per cent of them. It is conceivable that if blood were more often examined fresh in moist chambers, the condition would be found to be more common than it is supposed to be and to be present in other races than the negro.

The majority of persons whose blood contains sickle cells do not appear to be suffering from any effects of the condition; strikingly enough this cellular form seems to run in families. Emmel first observed this in 1917, and the observation has been extended and elaborated to such degree that Huck even said the condition followed the mendelian law and the character acted as a simple dominant. Of course this has yet to be demonstrated, since the literature does not yield enough to confirm such an assertion. If it were a dominant negro character it would hardly appear in such a low percentage of persons, since a simple dominant should appear in the long run in 75 per cent of individuals of that race. Certainly if this character is a part of an allelomorphic pair, some other explanation must be offered than that it is a simple mendelian dominant. Males are three times more frequently affected than females, yet no one has suggested that the character of sickle cell is sex linked.

Whether this condition of the erythrocytes tends to make one more susceptible to anemia is a matter for speculation, but anemia is usually the sign which prompts one to examine the erythrocytes and to disclose the phenomenon. The anemia often overtakes the victim in childhood or early adult life; the average age is twenty-three years. The patient may experience many exacerbations of the disease. Hahn and Gillespie offered as an explanation of the relation of the sickle cells to the anemia, that as a result of the reduction of oxygen tension by cardiac or pulmonary disease, the erythrocytes of susceptible persons assumed the sickle form. These cells are likely to be the victims of phagocytosis and other hemolytic agents, with resulting anemia. But Dolgopol and Stitt have expressed the belief that anoxemia caused by pulmonary disease does not have anything to do with the anemia.

Other observers have dealt with the original cause of the formation of

sickle cells. Emmel suggested that the cell was produced by accentuation of the normal hematogenetic activity which in persons who lack susceptibility to formation of sickle cells transforms the spherical erythrocyte into a biconcave disk. In persons who are susceptible to formation of sickle cells, the process goes further. Josephs stated that changes in surface tension in the blood elements are the foundation of the condition. Levy has held to the view that the sickle-shaped cell is an embryonic normoblast, hatched before its time, so to speak. Most workers admit some hereditary influence and this is as far as the matter has progressed toward settlement. However, the formation of such cells has been witnessed by several observers when blood from susceptible persons has been watched under suitable conditions. When blood containing sickle cells is transfused into a normal person, these cells may be seen to remain in circulation for several weeks, indicating to some extent their viability in foreign serum.

Most of the symptoms now known to be characteristic of this entity were observed by Herrick. Systolic murmurs are common, along with jaundice and adenopathy. The liver usually is enlarged. The spleen is not enlarged according to some observers, whereas others record finding enlarged spleens in a majority of cases. A curious phenomenon of the disease is the usual presence of ulcers of the leg. The ulcers are persistent and leave a permanent scar. The anemia is often severe, with the usual hematologic signs, and a low color index. The blood chemical values are not greatly altered, but an indirect van den Bergh reaction and a high proportion of bilirubin in the blood has been recorded. The presence of sickle-shaped erythrocytes distinguishes this anemia from all others and also from hemolytic jaundice.

The pathologic examination, except that it reveals the changes due to anemia, discloses a characteristic spleen, according to Rich. There is abnormal development of follicular capillaries, and malformation of the sinuses around the follicles, with presence of pools of blood in the pulp about the malpighian bodies. The bone marrow undergoes grave changes, and, according to Graham, sclerosis of the cortex and marrow occurs beneath ulcers of the leg; elsewhere the cortex is thickened, there are regions of hyperplastic marrow, and necrosis with intramembranous repair of the bone.

The prognosis, if based on the records of necropsy, would be generally favorable, for but few deaths seem to have been due to the disease itself. Yet there is general agreement that the condition makes it difficult to recover from intercurrent diseases, even more than is accounted for by the degree of anemia, which of course lowers the general vitality of the patient. As Steinberg well puts it, "A patient with sickle-cell anemia and some intercurrent disease is more apt to succumb to the secondary condition."

No form of treatment stops the development of sickle cells. Splenectomy, a kind of supposed cure-all for blood dyscrasias, is certainly of doubtful value in this disease. Transfusions give temporary relief and increase the number of erythrocytes but do not stop the hemolytic processes. Liver diets, as used by Levy, offer most in return for the effort expended in treatment and should be given an extensive trial in the light of his observations and of the general experience with liver in other forms of anemia.

Sickle cell anemia is a disease to whet the scientific appetite of both clinicians and laboratory men, for enough is known about it to point to the possibility of many fundamental contributions being made to the subject of blood dyscrasias through study of this condition.

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—*T. B. M.*

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THE CLINICAL LABORATORY AS AN AID TO SURGERY

A SYMPOSIUM

THE CLINICAL LABORATORY AS AN AID TO THE SURGEON

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THAT the evolution of modern surgery and surgical technic which now permit the surgeon to attack disease in the most vital recesses of the body; even to suggest entering which, a half century ago would have been to spell death to the patient and anathema to the surgeon, are the direct outcome and aftermath of laboratory research, is common to the knowledge of even the casual student of medical history.

That an ever-broadening understanding of the etiology and the mechanism of disease processes, the evolution and evaluation of new methods of treatment, and the accumulation of data relative to prognosis are all definitely related to and assisted by, if they do not arise directly from, laboratory studies, is likewise apparent to the most infrequent reader of current medical literature.

It is not the purpose of this symposium to recount or even to emphasize these self-evident facts, but rather to call attention to, and, if necessary, to emphasize the intimate relation and the practical value of the clinical laboratory to the everyday problems of the everyday surgeon; and to remind him that, now that it is obligatory upon accredited hospitals to furnish competent laboratory surveys under competent supervision, these are available in any hospital worthy of the name.

The experience of any pathologist, especially if he is not associated with a teaching institution, will corroborate the statement that, as a general rule, it is the medical rather than the surgical divisions which most consistently call upon the resources of the laboratory and the pathologist. Nor is this altogether to be marveled at, for, whereas, in the medical divisions the im-

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mediate and outstanding problem is essentially and primarily one of diagnosis, in the surgical divisions, the immediate question at issue is not so much what to do but how shall it be done?

In other words, the patient entering the medical division is largely an unknown and uncertain factor. His chief complaint may be most misleading; his symptoms vague, obscure, and puzzling; the whole picture presenting a puzzling problem the elucidation of which may lead through a sinuous, tortuous path and require all the skill and acumen, all the resources which the clinician may possess or which he can summon to his aid.

The patient entering the surgical division presents a problem one feature of which, at least, is, in the great majority of instances, fairly clear-cut: he is a surgical case. There may be room for discussion or debate as to when, or how to operate; where to cut, and what type of operation to perform; but, as a general rule, that he is primarily a case for operation is rather definitely established.

Under these circumstances it is not unusual to find that many surgeons fail to receive from the clinical laboratory the assistance it is often able to give, and that too many regard the preoperative uranalysis and leucocyte and differential count as the ultima Thule of its utility.

This is not to suggest that laboratory studies be given the first or paramount importance in the study of disease, but rather to claim for them their proper place as *one* of the avenues for the study of disease and its reaction upon the patient.

It is the fate of every development in the history of medicine, by which term is meant all that is included in the recognition and management of disease, to undergo, for a time, an undue accentuation of its importance, and all that is included in the phrase "clinical pathology" is no exception to this rule.

No one has been quicker than the clinical pathologist to pronounce against the current tendency to attribute undue importance and undue value to laboratory studies in disease, or to inveigh against a too common habit of regarding laboratory "tests" as the final, if not the only avenue of diagnostic approach.

It is particularly unfortunate that the procedures of the clinical laboratory should be so commonly thought of and referred to as "tests," as this term not infrequently seems to be accepted as implying a definite value to "positive" or "negative" determinations. This is far, indeed, from the purpose of laboratory methods and farther still from the real significance of their results.

There is nothing in the situation too complex for understanding if only the various factors concerned are given their true values.

The premises, in essence, are simply these:

First, that the recognition of disease depends mainly upon the recognition of its manifestations in the patient.

Second, that the reactions of the patient to pathologic stimuli are governed by and dependent upon, not only the nature of the stimulus and the manner and degree in which it is applied, but also, which is of vital importance, upon the ability of the particular patient to react.

If these premises be accepted, it is readily apparent and should be obvious that the methods of the clinical laboratory are *not* to be and cannot be regarded as "tests" for the presence or absence of disease, but rather as particular and specialized methods of examination, the end and purpose of which is to detect the presence, and measure the degree of certain and various reactions exhibited by the patient, reactions ascertainable by no other means.

The determination of the presence, or absence, of a particular reaction, be it leucocytosis or leucopenia; a "shift to the right"; an accelerated sedimentation time; a positive complement-fixation reaction, or what not, constitutes simply an established fact, an item in the array of information gathered from the various and varied studies in a particular case.

The value and the significance of this item of information, as is true, indeed, of *all* the information acquired by whatever means, is entirely dependent upon the acumen with which it is interpreted which, in turn, depends upon the background of past experience from which the interpretation arises and upon which it is based.

The laboratory procedure establishes and to a greater or less extent indicates merely the reaction or absence of reaction to pathologic stimuli; its true value and only value in the study of disease arises solely from the fact that it forms a starting point for inferential and deductive reasoning leading to the detection of the nature of the stimulus, to an estimation of the ability of the patient in question to respond to it, all matters of importance in the study of his disease.

The position of the clinical pathologist is based upon such concepts. He argues not for the unnecessary or unnecessarily extensive employment of laboratory methods; not that they shall be used merely to convey a pseudoscientific atmosphere; not that they shall supplant clinical studies or be accepted as replacing clinical acumen; but that they shall be recognized as a distinct and valuable *single* phase in the study of the patient and used intelligently to that end.

While this may appear to be a somewhat roundabout approach to the subject at hand, it can be justified by the experience of clinical pathologists in general, and, at least, serves to clear the way for more pertinent and concrete discussion.

It is not the purpose of this paper to discuss in detail any particular method of the clinical laboratory, but rather to generalize, to indicate in a broad manner some of the various ways in which the clinical laboratory may be utilized by the surgeon to his own and the patient's advantage.

In the field of surgical diagnosis, the outstanding contribution of the laboratory is, with little doubt, the histologic study of tissue, performed either before the operation, as in biopsy, after the operation when the tissue has been excised, or, as is becoming more and more feasible, during the operation by rapid methods, the results of which may be of value as indicating the appropriate surgical measures to be undertaken, for few will dispute the essential value of laboratory studies in the diagnosis and management of malignant neoplasms or, indeed, the study of tumors in general.

The presence of a surgical condition is one thing while its amenability to surgical interference, as well as the ability of the patient to permit and withstand such interference constitute another and a distinct and vital phase of the question, to the study of which the resources of the clinical laboratory may well be directed.

More than once has the humble uranalysis by its demonstration of glycosuria saved a patient from surgical procedures, simple in themselves but dangerous under the circumstances, and more than one patient has sunk into diabetic coma because the uranalysis was simply a "routine" procedure, the results of which were not consulted until after the operation.

Patients have been curetted for menorrhagia due to "hyperplastic endometritis" later shown by cytologic blood studies to be a manifestation of leucemia, and the tabetic crises operated upon under various guises but later recognized when the blood and spinal fluid were examined are familiar to us all.

The recent literature establishes beyond question the value and the necessity of laboratory studies in the diagnosis and management of diseases of the liver and bile passages; in fact, there is hardly a field of surgery or a surgical problem in which and to which the clinical laboratory cannot bring something of value.

The problem confronting the surgeon is often not merely one of diagnosis or of establishing the necessity for operation, especially when the operation is one of election rather than of necessity. Of equal importance is it to determine the suitability of the particular patient for operation; to determine whether he can be operated upon with a reasonable margin of safety; to learn whether the functional efficiency of his organic structure is able to withstand successfully the stress of surgical procedures or the strain of their aftermath; to ascertain whether or not concealed or unrecognized lesions, weaknesses, or hidden aftermaths of previous disease, may completely change the aspects of the situation.

In all of these situations the clinical laboratory may often be utilized to definite advantage, and while such studies may be of relatively minor importance in one case, in another their value and significance may be paramount.

What surgeon contemplates with equanimity the performance of a prostatectomy unless he is informed as to the functional efficiency of the kidneys? Who would knowingly operate upon a patient with extreme anemia unless first safeguarded by transfusion or preliminary treatment? Who would approach surgically a diabetic without some attempt at stabilization beforehand? And how may all these facts and factors be studied without laboratory aid?

Even such generalizations as these suggest the important function of the clinical laboratory in the preoperative study of the patient and in the recognition of both borderline as well as absolute surgical contraindications.

It is unfortunate that the methods and procedures of the clinical laboratory are so often thought of in connection with the diagnosis of disease and greater attention not more generally paid to their utility as a means of following the progress of disease by the detection and, to some extent, the

measurement of the reactions which occur in the body of the patient which, by their character and extent, often exemplify the old adage that "coming events cast their shadows before."

A sudden and marked indicanuria, for example, observed after operation may often be the first warning of a beginning peritonitis, just as a sudden postoperative disappearance of the urinary chlorides suggests the imminent possibility of a concealed focal or central pneumonia.

Half the battle against disease is won when the disease is recognized and the early institution of appropriate treatment thus made possible. This principle holds true under any circumstances but possesses, perhaps, a particular importance in the consideration of postoperative complications.

It is quite true that a postoperative rise in temperature may be due to toxic absorption, but, and especially when it is marked, sustained, or subject to suspicious fluctuations, it may also be due to bacteriemia. The question is readily settled by a blood culture and should be so settled at the earliest possible moment rather than left to the time when the culture shows innumerable colonies to the cubic centimeter of blood.

The bacteriologic methods of the laboratory have a most intimate and important relation, not only to the recognition of many surgical complications, but to the understanding of many surgical diseases as well as to the evolution and perfecting of methods for their arrangement.

It should not be considered sufficient to recognize or reluctantly admit postoperative infections. Much more to the point is to determine their nature bacteriologically and so, perhaps, to bring to light minor breaks in technic, not to mention the exoneration of the so often automatically incriminated catgut.

Nor is it without value, from time to time, to subject the operating room, its methods and its personnel to bacteriologic survey and control.

Not without value and utility also are bacteriologic as well as histologic surveys of excised tissues or organs, culture of empyema fluids, cultures from infected wounds and the like, for if these do not stimulate the imagination at least they result in the accumulation of data for some other, later, and more active mind to ponder upon in the future.

There would appear to be at times a tendency to overlook the fact that one, and not the least important, of the functions of a hospital is, or should be, so to study and analyze the clinical material which falls to its lot that something, no matter how little, may be added to the sum total of the understanding of disease.

An honest attempt at the collection, correlation, and analysis of information is never entirely unproductive. If nothing is added thereby to general knowledge, something will nevertheless accrue to the knowledge of the student.

To many the idea of such studies predicates the idea of "research," and, unfortunately, research is regarded by too many as the special prerogative of large, extensively equipped, and expensively endowed institutions manned solely by "Professors" with teaching affiliations.

This concept, of course, is not only erroneous but conclusively refuted by medical history which abounds in contributions, often of the highest value and importance, which had their inception, in the conscientious work of individuals whose most important equipment was their enthusiasm, their imagination, and their dogged persistence.

These things are possible to any institution and may justly be demanded of those who conform in personnel and equipment to the standards for hospital approval.

Equipment in itself is nothing unless there be also the will to utilize it to the best advantage.

Let there be but one, and it matters little whether he be internist, surgeon, or pathologist, who has enthusiasm, who is gifted with the spirit of inquiry, who is willing to expend energy, thought, and labor in the collection and analysis of data, and results are inevitable, for the flame of enthusiasm once kindled, must spread. Great discoveries are more often the result of chance than deliberate plan, of persistence rather than inspiration, and while it may be given to but few to make them, it is within the reach of many to add *something* to human knowledge.

In the hands of internist, surgeon, and pathologist working together lies the medicine of the future and great is their responsibility, if, for lack of cooperation, inability or disinclination to coordinate their abilities, or casual or deliberate disregard of their interlocking functions, they let the opportunity pass.

BLOOD PATHOLOGY IN RELATION TO SURGERY*

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THIS subject can be taken up under four heads: (1) diseases of the blood and hematopoietic organs, in which operation is indicated or must be considered; (2) surgical diseases of other types, in which study of the blood may be of value in diagnosis, prognosis, or treatment; (3) hemorrhagic diseases as possible contraindications to operation; and (4) postoperative complications in surgical cases in which studies of the blood are of value.

Diseases, the diagnosis of which depends wholly or partly on chemical and bacteriologic studies of the blood and not on studies of the morphology of its formed elements or on studies of its mechanism of coagulation, are not considered in this paper. Only the more serious diseases can be taken up because of lack of space; for the same reason, the statements made must be more or less dogmatic, devoid of theoretic considerations, and to many may appear elementary.

DISEASES OF THE BLOOD AND HEMATOPOIETIC ORGANS IN WHICH OPERATION IS INDICATED OR MUST BE CONSIDERED

The spleen and lymph nodes are commonly considered as belonging to the hematopoietic apparatus. In any case in which there is considerable enlargement of the spleen or lymph nodes, the question must arise as to whether some surgical condition exists. An exhaustive study of the results of splenectomy in 530 cases has recently been made by W. J. Mayo and an earlier study of this sort was made by Giffin. More exhaustive diagnostic data than can here be given can be found in the paper by Giffin and in another paper, by me.

The three most common conditions of the spleen, in which operative procedures are indicated, are congenital or acquired hemolytic icterus, purpura hemorrhagica, and splenic anemia. Rarer conditions for which operation usually is required are tumor and cysts of the spleen, tuberculosis of the spleen, and syphilitic spleen, when the size of the spleen has not responded well to antisyphilitic treatment. Other conditions of the spleen for which surgery is sometimes considered are tuberculosis of the spleen, Gaucher's disease, splenomegaly accompanying some cases of cirrhosis of the liver, myelogenous leucemia, von Jaksch's disease, and infectious splenomegaly of indeterminate type accompanied by focal infection. A malarial spleen is not often removed if the correct diagnosis is made, and the same is true in splenomegalic polycythemia.

In hemolytic icterus the increased fragility of the erythrocytes, increase in number of reticulocytes, tendency to slight leucocytosis, increased con-

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centration of bilirubin in the serum, indirect van den Bergh reaction, marked polychromatophilia, and, in the congenital type, the well marked general microcytosis, are the distinguishing features of the blood.

In purpura hemorrhagica the diagnosis depends largely on examination of the blood. The distinguishing features are as follows: very low values for platelets, long bleeding time, normal coagulation time of the venous blood, and prolonged time of clot retraction. Its distinction from aplastic anemia, with which it is so often confused, to the great chagrin of the surgeon, depends on the presence, in aplastic anemia, of marked anemia, unaccounted for by the hemorrhage, numerically deficient or absent reticulocytes, leucopenia and relative lymphocytosis, in addition to those changes found in purpura hemorrhagica. I have seen a considerable number of cases in which distinction between these two diseases has been difficult. It seems likely that some of the fatal results reported after splenectomy for purpura hemorrhagica may be due to failure to make this distinction. Likewise, acute leucemia must be distinguished from purpura hemorrhagica by the presence of immature leucocytes in the blood. Hemophilia differs from purpura hemorrhagica in that in hemophilia there are prolonged coagulation of the venous blood, normal bleeding time, a normal number of platelets, normal clot retraction time after the clot forms, and prolonged prothrombin time of Howell. The simple purpura and that of Schönlein and Henoch are not accompanied by any definitely diagnostic changes in the blood.

Splenic anemia, for which splenectomy is the recognized treatment, is probably not an entity. However, in a case presenting splenic enlargement of considerable degree, gastric or intestinal hemorrhages, leucopenia, reduction in number of erythrocytes and in hemoglobin, low hemoglobin index, and relative lymphocytosis, the diagnosis seems warranted. To those cases of similar nature, accompanied by ascites and cirrhosis of the liver, the designation Banti's disease usually is given.

In the presence of tumors or cysts of the spleen there are no significant changes in the blood except when echinococcus cyst is present. This condition may be accompanied by eosinophilia, and the blood serum usually gives a positive complement fixation of Weinberg; the fluid of an echinococcus cyst is used as antigen.

Tuberculous splenomegaly is not accompanied by significant changes in the blood, and syphilitic splenomegaly is not accompanied by any except the characteristic serologic evidence; diagnosis must depend on finding evidence of these diseases, elsewhere. I recently saw a case in which very severe purpura hemorrhagica was accompanied by tuberculous spleen.

In Gaucher's disease there are no diagnostic changes in the blood, although anemia usually is present. The diagnosis of Gaucher's disease depends on the following features: it appears principally in patients of Jewish origin, it has a familial tendency, there is pigmentation of the skin, the spleen is of enormous size, there is a wedge-shaped pad of fat on the sclera, and evidence of changes in the bones are seen in roentgenograms.

There are no significant features of the blood in the presence of splenomegaly accompanying cirrhosis of the liver, or in infectious splenomegaly.

The Rowntree-Rosenthal dye test of hepatic function may help in the diagnosis of cirrhosis of the liver.

Myelogenous leucemia, of course, depends mainly for its diagnosis on the presence of leucocytosis and a high proportion of myelocytes and the presence of younger forms of granular leucocytes. However, considerable value is attached to the presence of an increased number of eosinophils or basophils, and to an increase in the number of platelets. Occasionally the chief manifestation of lymphatic leucemia is splenomegaly, and here, too, the leucocytosis and the high percentage of lymphocytes allows the diagnosis to be made. Care must be taken not to overlook those cases of leucemia in which the total number of leucocytes is normal or decreased but the differential count is characteristic of leucemia. A decreased or normal number of leucocytes is found, however, more often in acute leucemia, which often is not accompanied by splenomegaly or enlargement of lymph nodes. One must emphasize the value, in doubtful cases, of frequent study of blood smears in any case in which leucemia is to be considered.

Von Jaksch's disease, which occurs in infancy, is characterized by marked splenomegaly, usually accompanied by high leucocytosis and a relative increase in the proportion of polymorphonuclear cells.

In diagnosis of disease accompanied by splenomegaly malarial spleen must be considered also. This is identified at times by the malarial plasmodium in the blood, the leucopenia, and the tendency to mononucleosis. Kala-azar, seen in Egypt, Assam, Indo-China and some other localities, must be considered; it is identified by the Leishman-Donovan bodies in the blood and in material obtained by splenic puncture.

In the splenomegaly of so-called splenic Hodgkin's disease these are no absolutely diagnostic features of the blood. Bunting, however, has laid emphasis on the occurrence of an excess of monocytes in the blood, especially the monocytes commonly called transitional cells.

The diagnosis in splenomegalic polycythemia depends on the red or bluish-red skin (especially of the face, lips and tongue), the splenomegaly, the increase in number of erythrocytes and percentage of hemoglobin, the increased viscosity of the blood and the increased blood volume. Moreover, leucocytosis usually is found.

Principal diseases causing enlargement of the lymph nodes to such an extent that operation might be considered are as follows: lymphatic leucemia, Hodgkin's disease, lymphosarcoma, metastatic carcinoma and sarcoma, tuberculosis, syphilis, and chancroid.

In none of these are there significant changes in the blood except in syphilis, leucemia, and possibly Hodgkin's disease, all of which have been considered.

SURGICAL DISEASES OF OTHER TYPES IN WHICH STUDY OF THE BLOOD MAY BE OF VALUE IN DIAGNOSIS, PROGNOSIS, OR TREATMENT

In this group, the leucocyte count is probably of prime importance. In fact, next to examination of tissue and of urine, it is probably the laboratory procedure of greatest value to the surgeon.

Its main importance lies in the diagnosis of infection. On the whole, the number of leucocytes is greater in the presence of purulent infections than in the presence of nonpurulent infections and in the presence of free rather than of well walled-off abscesses. Leucocytosis is greater if abscesses are not drained than it is if they are drained. The presence of leucocytosis does not necessarily indicate the presence of pus, nor does the absence of leucocytosis surely indicate the absence of purulent material. In the presence of obvious infection, a high leucocyte count indicates a severe degree of infection, or good resistance on the part of the patient. Mild leucocytosis indicates a lesser degree of infection or poor resistance to it. Sondern has expressed the belief that the height of the leucocytosis is a measure of the resistance of the patient, whereas the percentage of polymorphonuclear neutrophils indicates the degree of virulence of the infection. Certain it is that a low value for leucocytes, in the presence of severe infection, ordinarily accompanied by high leucocytosis, is of serious prognostic omen.

It is certain, also, that the differential leucocyte count is of the greatest value. Serious infection may be indicated by normal values for the total number of leucocytes accompanied by large increases in the percentage of neutrophils. Neutrophilic values of 80 to 85 per cent often are found in the presence of severe infections in which the total number of leucocytes may be normal, subnormal, or only slightly increased.

Arneth attempted to give greater diagnostic and prognostic value to the enumeration of leucocytes by estimating the relative percentages of various types of neutrophils. Because of the laborious procedure involved, this has never met with great favor, in spite of its value.

Schilling's hemogram consists principally of a method of estimation of the percentage of neutrophils that have, respectively, segmented, rod-shaped, indented, or round nuclei. Those with indented nuclei he termed young neutrophils; those with round nuclei are the well-known myelocytes. Schilling also included in his hemogram estimation of the proportion of eosinophils and basophils, monocytes and lymphocytes. The essential thing in the interpretation of the hemogram, so far as infection is concerned, is the degree of the "shift to the left." The shift to the left is indicated by an increased number and percentage of cells with rod-shaped nuclei, young forms and myelocytes, and a decreased number and percentage of those with segmented nuclei; the severer the infection the greater the shift to the left, and the smaller the percentage of eosinophils. The Schilling hemogram bids fair to become a valuable procedure in diagnosis and prognosis.

In interpretation of leucocytosis one must bear in mind the normal increase in number of leucocytes under the following circumstances: as the day wears on; during the digestive period; after exercise; during pregnancy; after a cold bath, and after administration of certain medicines such as quinine and camphor. One must also remember the mild leucocytosis which accompanies malignancy and the leucocytosis usually found as a consequence of splenectomy. The number of leucocytes is likely to be greater in childhood than in adult life as a result of the same stimuli. The increase in total number of leucocytes in childhood, however, is more likely to involve a pro-

portionately smaller percentage of neutrophils than in adults. In the diagnosis of the cause of acute upper abdominal or thoracic pain, one must keep in mind the leucocytosis that occurs as the result of coronary occlusion. Furthermore, the leucocytosis following severe hemorrhage must constantly be remembered. The value of toxic granulation of the neutrophils is not yet well established, but further study may show it to be of considerable consequence. Thorough study of the blood smears should be made in any doubtful case of infection or anemia.

In malignant disease involving any organ, a picture somewhat resembling that found in myelogenous leucemia, may occur as the result of metastasis to the bone marrow. Recently there has come under my observation at The Mayo Clinic a case of metastasis to the bone marrow from a carcinoma of the stomach in which there were about 10 per cent of myelocytes, and the total number of leucocytes in each cubic millimeter of blood was 32,000.

Eosinophilia may be of value in the diagnosis of parasitic infections such as echinococcus disease, trichinosis, amebic dysentery or abscess, and of abdominal pain of allergic origin.

Leucopenia may assist in distinguishing typhoid or paratyphoid fever from other causes of abdominal pain, and malaria from septic causes of chills and fever.

Estimation of the concentration of hemoglobin and of the number of erythrocytes is of value in surgery mainly in the recognition of the forms of secondary anemia caused by surgical diseases, such as carcinoma of the esophagus, stomach, intestine, rectum, vagina, uterus, kidney, or bladder, in which the anemia may be the result of hemorrhage or a part of the cachexia; hemorrhage from ulcer of the stomach, duodenum, or from the remainder of the intestine, including the rectum; bleeding from hemorrhoids, and hemorrhage from benign tumors of the stomach, intestine, rectum, uterus, bladder, and renal pelvis. A frequently overlooked source of secondary anemia is the small intestine, the condition of which cannot be successfully determined roentgenologically. A number of instances of surgical disease of the small intestine have come under observation at The Mayo Clinic in which the only evidence of disease of the small intestine was the anemia and the occult blood in the stool. W. J. Mayo has called attention to anemia as the chief evidence, at times, of carcinoma of the cecum.

Other causes of secondary anemia, such as infection and dietary deficiency, must be kept in mind also. The estimation of the concentration of hemoglobin, and of the number of erythrocytes, may be of value in determining serious acute hemorrhage, as in ruptured ectopic pregnancy or aneurysm. It should be borne in mind, however, that for several hours after the hemorrhage, because of the slow dilution of the blood by the body fluids, the amount of hemoglobin and the number of erythrocytes may not be reduced for each unit volume of blood, whereas the total values of both may be tremendously decreased.

Pernicious anemia often enters into consideration in the diagnosis of surgical conditions. The principal features disclosed by examination of the blood of patients with pernicious anemia are marked reduction in the con-

centration of hemoglobin and number of erythrocytes, high hemoglobin index and volume index, macrocytosis, anisocytosis, decreased number of platelets, and the presence of an increased number of lobules in the nuclei of the neutrophils. Mention should be made of the combination of pernicious anemia with some surgical disease, such as carcinoma of the stomach, intestine, or rectum, benign tumor of the stomach, or other acute or chronic surgical condition. Basophilic stippling of the erythrocytes may help to distinguish intoxication caused by lead from other causes of abdominal colic. I have seen a case of intoxication caused by lead in which there had been many abdominal operations for colic. In this case there was marked stippling of the erythrocytes.

The blood platelets are of value in surgery principally in aiding the diagnosis of the various hemorrhagic diseases and in determining the activity of the bone marrow.

The estimation of blood volume has few uses in surgery. Its greatest value is in shock, in which the blood volume is markedly reduced, and in the diagnosis of polycythemia vera, in which it is increased, sometimes greatly.

In surgery the sedimentation test of Fahraeus is mainly of value in the prognosis of tuberculosis and in the determination of pregnancy. A markedly increased rate of sedimentation is found in a high percentage of cases of severe tuberculosis and of pregnancy.

The complement-fixation test of the blood has its greatest value in syphilis in the form of the Wassermann test and its various modifications, in echinococcus disease, in which a positive test is found in 80 to 90 per cent of cases, and, if found, is almost certain indication of the disease, and in gonorrhea and tuberculosis, in which its value is not so well proved.

Agglutination tests, made with the patient's blood, are of value in surgery mainly in the form of the Widal test for typhoid and paratyphoid fever and as the agglutination test for undulant fever and for tularemia.

Examination of the blood for parasites is of interest to the surgeon in some cases. His interest in finding the organism of malaria is not confined to explaining enlargement of the spleen, which has been mentioned, but extends to finding an explanation of chill and fever of obscure, but suspected septic, cause. Occasionally latent malaria may become active after surgical operation. The surgeon's interest in kala-azar has been pointed out. *Filaria sanguinis-hominis* must be searched for in the blood of patients with elephantiasis, especially in those from India. Trichinae are found in the blood only during the stage of migration of the parasite. The tremendous eosinophilia, and the discovery of the trichinae in a piece of excised muscle, are much more reliable diagnostic indications of this disease.

HEMORRHAGIC DISEASES AS POSSIBLE CONTRAINDICATIONS TO OPERATION

In any case in which a surgical procedure is contemplated, one must make sure that hemorrhagic diathesis is absent. The principal conditions associated with a hemorrhage tendency are hemophilia, the several forms of purpura, and the hemorrhagic tendency which appears in obstructive jaundice.

Hemophilia is distinguished by its peculiar hereditary tendency, its oc-

currence in the male only, its transmission through the female only, the occurrence of massive hemorrhages in the joints in addition to those elsewhere, the long coagulation time of the venous blood, the long prothrombin time of Howell, the normal number of blood platelets, and the normal clot retraction time after clotting occurs.

Purpura hemorrhagica is characterized mainly by the occurrence of purpuric areas on the skin and mucous membranes, long bleeding time, prolonged clot retraction time, normal coagulation of the venous blood, decreased number of platelets, and the positive capillary resistance (or positive "tourniquet") test.

From the standpoint of examination of the blood, Schönlein's and Henoch's purpura, and purpura simplex, are characterized by a normal number of platelets, normal bleeding time, and normal clot retraction time. Usually, also, coagulation time and the prothrombin time of Howell are normal.

In cases in which there is hemorrhage from the mucous membrane of the stomach, intestine, kidney, urinary bladder, or uterus one must make sure that none of these hemorrhagic diseases is the cause of the hemorrhage before operation is undertaken.

It is necessary, before operation is performed, to determine the coagulation time of the venous blood in all cases in which there is jaundice.

Attention must be called to certain cases in which a definite diagnosis cannot be made of the exact type of hemorrhagic disease that is present. In any case of hemorrhagic disease, operation should be attempted with the greatest caution, if at all. This is particularly true in hemophilia, although Minot has stated that minor operations often can be performed with justifiable risk, if transfusions of large quantities of blood are given before and after the operation. These transfusions should be sufficiently large to keep the coagulation time of the blood at a low value during the operation and during the healing process. Operation for true purpura hemorrhagica, however, is of curative value, at least so far as the symptoms are concerned.

POSTOPERATIVE COMPLICATIONS IN SURGICAL CASES IN WHICH STUDIES OF THE BLOOD ARE OF VALUE

In postoperative complications, the leucocyte count is of preeminent value among all laboratory procedures. Here, again, its value consists in the aid it gives to detection of infection, either as a result of the operation or as a part of a separate disease occurring during the postoperative period. The reduced blood volume in postoperative shock may be of value in its diagnosis. The number of erythrocytes and the concentration of hemoglobin may be reduced in anemia resulting from operative or postoperative hemorrhage, or from infection. Also, if these values are low, they may indicate preexisting anemia or independent anemia, such as pernicious anemia, developing after operation.

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THE BLOOD PICTURE IN INFECTIOUS DISEASE*

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WHILE the study of the blood has been gradually receiving more and more attention in the past twenty-five years among hematologists, the value of the morphologic blood picture in infectious disease has slowly ceased to impress both the physician and surgeon at large. A careful investigation into the probable causes that underlie this gradual disinterest, reveals the following: Before more thorough morphologic blood studies were made, the universal teaching had been that the higher the percentage of polymorphonuclear neutrophils, the severer the infection, while the greater the number of leucocytes, the higher the patient's resistance. Employing this dogma as the central principle in the diagnosis and prognosis of acute infectious disease, it immediately became evident that the discrepancies would be manifold. Thus the clinician who expected to find a marked leucocytosis in some case of pneumonia or some other condition with hyperpyrexia would be chagrined to find a normocytosis or even leucopenia, whereas the surgeon seeking hematologic aid in the differential diagnosis of several abdominal conditions, would be in a similar quandary. Ofttimes when the blood picture showed either a normal or slightly increased leucocyte count with an unconvincing increase in neutrophils, operation would reveal advanced disease of the suspected organ. Thus this large section of the profession in their effort to correlate the laboratory and clinical findings soon felt that blood examinations were of relatively little assistance. Added to this group we have a smaller part of the profession who in their enthusiasm for laboratory procedures have sought to raise the blood picture to the level of a pandiagnosticum. When the latter realized that the blood morphology could not differentiate various medical from surgical infections, their interest lagged and allowing the pendulum to swing to the other extreme actually felt that blood examinations were useless. The object of this paper is to demonstrate the basic value of the blood picture in both medical and surgical conditions.

Though Ehrlich was able by means of his blood stains to differentiate and classify the various leucocytes of the circulation, it was not until the appearance of Arneth's publications that the scrutiny of the individual leucocytes and their nuclear structure was attempted. While working in the Leube Clinic he made a series of observations which established definite relationship between the presence of an infection and the number of nuclear segments in the neutrophils. Varying proportionately with the severity of an infection the number of segmented neutrophils disappeared from the circulation, being replaced by nonsegmented forms. With convalescence and recovery the normal relationship is reestablished. Having subdivided the neutro-

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phils by means of the shape and number of nuclear segments, Arneth sought to do the same for all other leucocytes. Schilling and others, finding these numerous subclasses time-consuming and of very little practical importance, suggested that all neutrophils with more than one lobed nucleus be included under the heading of Segmented. All nonsegmented forms were to be divided into Jungkernige and Stabkernige (staff form). These cells differed inasmuch as the first had a plump kidney to sausage-shaped nucleus, whereas the latter was thinner and showed evidence of forthcoming segmentation by its varied and numerous contorted forms.

In addition to the study of the neutrophils during infection, the other leucocytes have of late also received quite a bit of attention. Careful serial blood studies have shown that the leucocytic elements of the circulation reflect

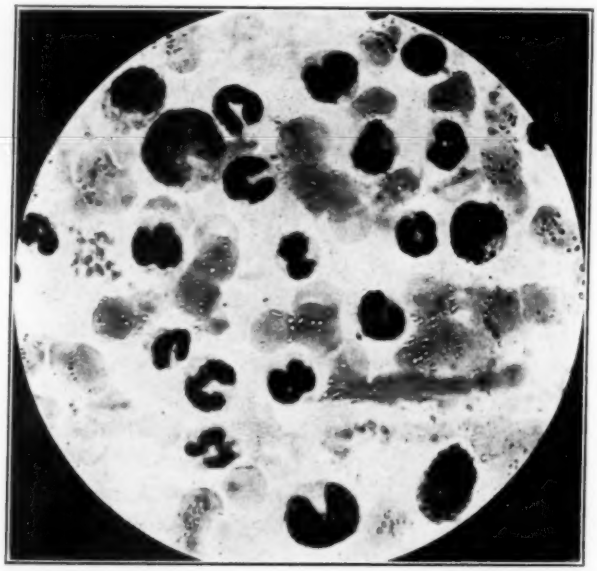

Fig. 1.—Showing a blood smear and a marked predominance of immature neutrophils. Majority of the leucocytes are staff cells. There are two metamyelocytes.

to a certain extent the reaction of the human organism to the invasion by a microorganism. Observers have demonstrated that the relative and absolute variations in the blood scheme were not due to the increase and decrease of neutrophils but to a hyperaction of each of the three cellular systems at a more or less specified time. Thus we have the neutrophilic phase or the period of combat. At this time there is marked evidence of neutrophilic hyperactivity in an effort to destroy the invading organism and its toxins. At the peak or climax of this period comes the monocytic phase. This interval is evanescent and usually denotes the defeat of the invader. Because of the shortness of this second period, it is often overlooked. Following this comes the final healing or lymphocytic phase. As a rule these three periods are not essentially clear cut. In chronic and subacute conditions we find an admixture of all three phases. Thus we see a high staff count associated with an increase of monocytes and

lymphocytes. We can therefore interpret the presence of increases in the three cellular components as an indication of a continued infection.

During the past five years we have had the opportunity of studying many thousands of blood specimens from both medical and surgical conditions. In our efforts to follow carefully this biologic chain of cellular interaction, frequent and serial blood examinations were made. Daily and often every six hours examinations were deemed necessary. Of these I have but chosen a small number of medical and surgical conditions most frequently found.

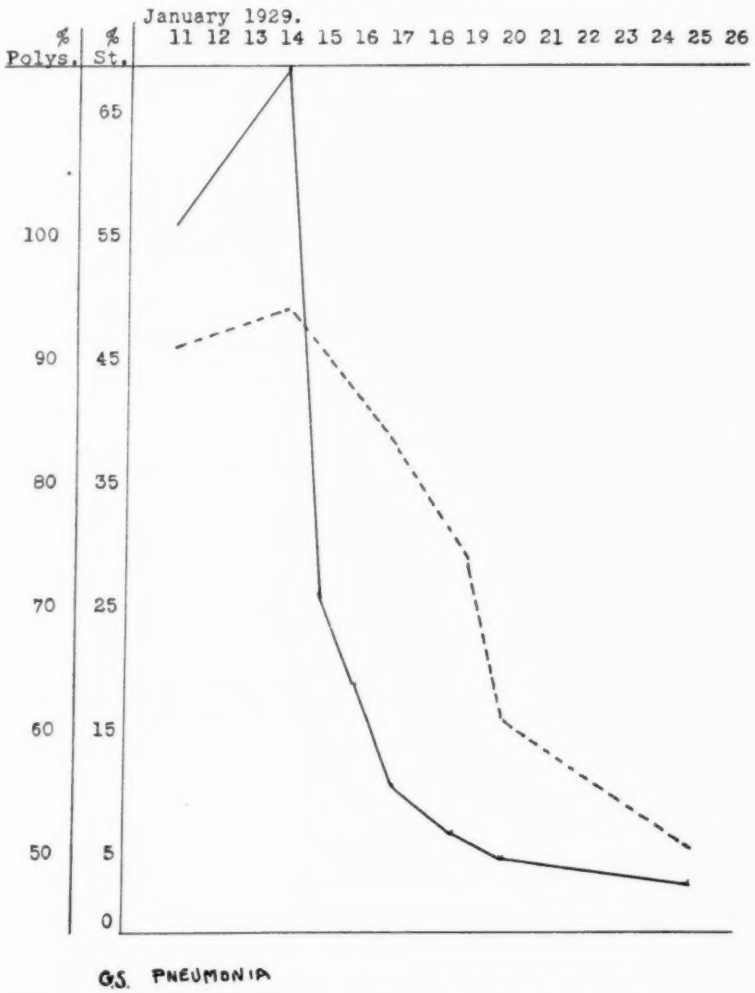

Chart 1.—Case 1. Shows the precipitate rise and fall of the staff curve. The neutrophilic curve though similar is less marked.

Case 1.—Miss S., aged twenty-one, was admitted on the medical service complaining of cough, fever, and right-sided chest pain. The patient had retired complaining of lassitude and slight headache. During the night she was aroused from a deep slumber by a sudden sharp lancinating pain in the right side of the chest. At that time she found that she had fever and that she breathed with difficulty.

Physical examination showed a very sick female of fair development. General examina-

TABLE I

SHOWING A SHARP RISE OF THE STAFFS TO 70 PER CENT AND THEIR IMMEDIATE DROP AFTER THE CRISIS. LEUCOCYTIC VALUES SHOW NOTHING UNUSUAL

	BLOOD															
DATE	ERYTHROCYTES NO. IN C.MM.	HB. PER CENT	INDEX	LEUCOCYTES NO. IN C.MM.	TOTAL PER CENT		POLYMORPHONUCLEAR					MONONUCLEAR			PLATELETS	ABNORMAL FORMS
					POLY.	MONO.	MET.	STAF.	SEG.	BAS.	EOS.	L	S	MON		
1/11	4,500,000	80		15,200	92	8		57	35				8			
14	4,060,000	65		10,200	95	5		70	25				5			
15				16,200	91	9		27	64				8	1		Marked degeneration of neutrophils
16				18,600	89	11		21	68				8	3		
17				18,200	86	14	2	12	74				4	8		
18				21,000	75	25		9	66				17	8		
20				14,500	62	38		6	53		3		36	2		
25				11,200	53	47		3	47		1		43	4		

tion was negative except for local chest signs. There were fine moist rales and pleural adventitious sounds. There was diminished and distant respiratory murmur over the right chest anteriorly. Breath sounds were bronchovesicular. Four days later tubular breathing was elicited over the right chest anteriorly and posteriorly. Heart sounds were regular and somewhat distant. On admission the temperature was 105.4°, pulse 128 and respiration 30. During her illness her pulse rate rose as high as 150. For a period of one week the patient looked very toxic and the prognosis seemed definitely fatal.

Laboratory data: Urine, sp. gr. 1010-1036 trace of albumin, few white blood cells. X-ray showed consolidation at the right base with compensatory emphysema of the left lung. Blood counts; see Table I. This case of pneumonia was of marked interest because the patient became extremely toxic early in her illness and continued to grow worse as the illness approached the crisis. The prognosis even early in the disease seemed hopeless. From Table I we notice that whereas the leucocyte count was but slightly increased, the percentage of neutrophils, and especially the percentage of staffs was extremely high (70 per cent). In spite of the fact that the clinician's prognosis, which because of the patient's marked toxicity, could only be bad, repeated blood examinations soon showed a gradual drop of the percentage of staffs indicating recovery of the paitent. Chart 1 shows the parallel rise and then drop of the staffs and neutrophils. Another point of marked importance can be gathered by examining the counts of January 11, 14, and 15. Though the leucocyte counts of these days showed nothing remarkable, the differential count of the eleventh, the day preceding the highest level of the staffs, and of the fifteenth, the day following the peak, showed the same number of neutrophils (90 per cent), as did the count of the fourteenth. Had the structure of the individual neutrophil not been studied, we should have overlooked the definite increase of the immature cells at the crisis, and their immediate drop thereafter.

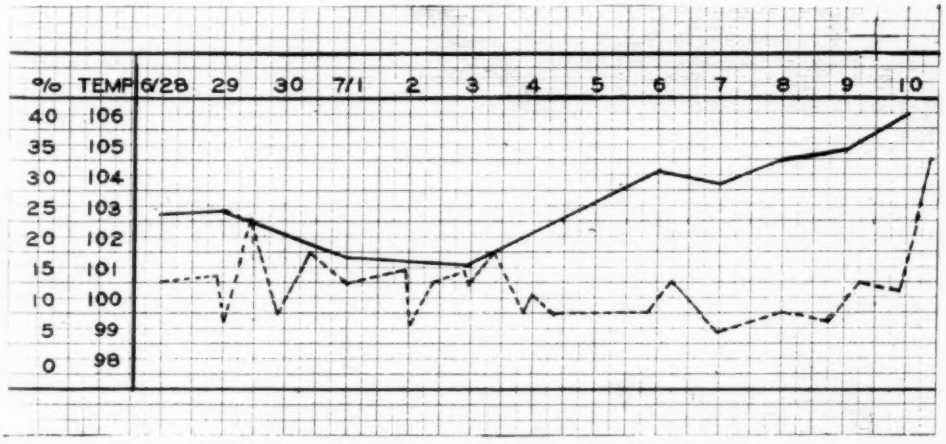

Chart 2.—Case 2. Shows a marked rise in the staff curve even while the temperature curve was low grade.

CASE 2.—Mr. W., aged sixty, entered the medical service complaining of right-sided pain in the chest, chills, fever, and cough. Physical findings were those of a bilateral bronchopneumonia. During the patient's first week in the hospital, he developed tympanites and retention of urine. With the clearing up of these complications the patient looked clinically improved. Blood examinations done during the latter part of his sojourn showed, in spite of the marked clinical improvement, a continually elevated level of the staff neutrophils. Although the clinician argued for a prolonged illness with eventual recovery, Table II and Chart 2 showed definitely the improbability of improvement. Autopsy of the patient revealed many small abscesses in the lungs. Chart 2 shows the steady rise of the staffs even when the temperature and clinical picture showed no such indication.

TABLE II

SHOWING A MARKED INCREASE OF METAMYELOCYTES AND STAFFS WHICH RISE TO THEIR PEAK JUST BEFORE DEATH. (THE FIRST FIVE COUNTS WERE DONE WITH OLD CLASSIFICATION.)

DATE	R.B.C.	HB.	W.B.C.	B.	E.	M.	MT.	ST.	S.	LY.	MO.
June 7	4,100,000	75	14,300						90	9	1
10	4,300,000	75	20,200						86	11	3
11	4,500,000	75	17,500		1				86	11	2
13			20,000						91	8	1
17			18,000						89	8	3
28			18,500				2	24	54	17	9
29			15,800				3	23	51	14	9
July 1							3	16	54	24	2
3			17,000				3	15	63	15	4
6							5	28	48	18	1
7							2	29	43	21	5
8							5	30	39	21	5
9			13,400				3	33	46	16	2
10							2	42	41	14	1

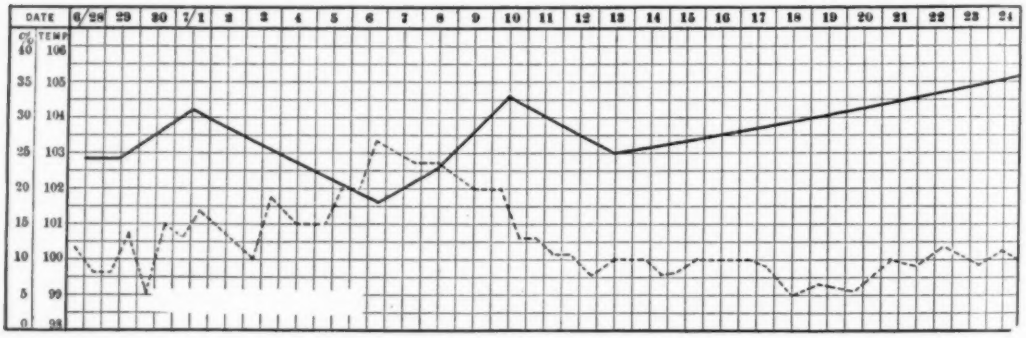

Chart 3.—Case 3. Shows high constant level of the staff curve with the temperature usually low grade.

TABLE III

SUBACUTE BACTERIAL ENDOCARDITIS (CASE 3). SHOWING PERSISTENCE OF A HIGH LEVEL OF IMMATURE NEUTROPHILS WHICH RISE TO THEIR HEIGHT BEFORE DEATH

DATE	R.B.C.	HB.	W.B.C.	B.	E.	M.	MT.	ST.	S.	LY.	MO.	
June 27	2,900,000	55	6,800					24	51	21	4	Blood culture, Strep. vir.
29	2,960,000	45	9,400					24	60	14	2	Neutrophils degenerated.
July 1	3,500,000	60	10,400				3	28	48	19	2	
6								18	56	18	9	Neutrophils degenerated; monocytes vacuolated.
8							2	21	55	15	7	Neutrophils degenerated; monocytes vacuolated.
10							1	32	44	15	7	Neutrophils degenerated; monocytes vacuolated.
18							1	24	35	35	5	Neutrophils degenerated; monocytes vacuolated.
24								36	40	20	3	Neutrophils degenerated; monocytes vacuolated.

CASE 3.—Mrs. H., aged thirty-four years entered the hospital on March 30 and died on July 25. She had been an old rheumatic cardiac with two previous hospital admissions. All laboratory data during her previous stays were negative including numerous blood cultures. On her present admission she complained of fever, headache and vague generalized pains. Blood culture on repeated occasions showed Streptococcus viridans. Table III shows a constant high level of staff cells. Neutrophils show marked degeneration as seen by irregular clumps of granules and irregular staining of the nucleus. Monocytes show marked hyperfunction as evidenced by numerous large and small vacuoles in the protoplasm. Chart 3 shows a constant high level of the staff cells with a gradual rise to its height just before death. It is of interest to note the low grade temperature which accompanied the terminal rise.

CASE 4.—Miss G., aged four years entered the hospital complaining of swelling of the legs, puffiness of the face and scantiness of urinary output. Urine had 4-plus albumin, hyaline and granular casts and many white blood cells. Temperature was intermittent, rising daily to 106° F. for a few days, and then dropping and remaining normal for a short interval. With rest in bed, restricted diet, and purgation all evidence of edema disappeared, but the intermittent temperature continued. With the administration of hexylresorcinol, the temperature returned to normal and the staff count reached its normal level. Table IV shows a marked increase in leucocytes with a staff count which rose as high as 17 per cent. With the clearing up of the underlying condition, the staff count dropped to 1 per cent.

TABLE IV

NEPHRITIS WITH PYELITIS (CASE 4). SHOWING A RISE IN THE NUMBER OF IMMATURE CELLS WHICH ALWAYS PRECEDES AND OUTLASTS THE PERIOD OF INCREASED TEMPERATURE

DATE	R.B.C.	HB.	W.B.C.	B.	E.	M.	MT.	ST.	S.	LY.	MO.
June 19	3,750,000	60	25,600				2	13	55	20	10
27	3,300,000	55	25,800					12	68	14	6
29			29,700					9	74	13	4
July 1	3,520,000	53	38,200				2	7	61	27	3
2			23,000				2	7	70	16	5
3			38,000					14	66	20	
6							1	16	48	34	2
7			23,400				3	5	48	35	9
8					1			9	67	16	7
15			15,700		1		1	3	60	35	
18	4,000,000	64	10,500		1			5	50	44	
30	3,760,000	60	15,600						55	42	2

The following cases are a few of the outstanding types to be found on any surgical service. A careful examination of these reports can but help to emphasize the definite place of close morphologic scrutiny in surgical diagnosis and therapy.

CASE 5.—Miss B., aged five and a half years entered the pediatric service complaining of fever, marked tenderness, and pain in the right lower extremity, especially the hip. In spite of a count of 23,300 leucocytes and 24 per cent staffs the consulting surgeon considering the case one of rheumatic arthritis, advised rest to the affected limb by means of an extension splint. As Table V well illustrates, the percentage of staffs remained high until evacuation of pus by incision of the right hip, was effected. After a slight reactionary rise, the immature neutrophil count rapidly dropped to normal.

TABLE V

INFECTIOUS ARTHRITIS (CASE 5). SHOWING THE PROTRACTED COURSE OF A PURULENT ARTHRITIS. STAFFS REMAINED HIGH UNTIL ALL PUS FOCI WERE EVACUATED

DATE	R.B.C.	HB.	W.B.C.	B.	E.	M.	MT.	ST.	S.	LY.	MO.	
July 19	4,720,000	70	23,300					24	67	9		
20	4,800,000	65	18,600					29	55	16		
23			25,600					28	57	15		Deg. of neutrophils.
26	4,000,000	65	38,200					17	64	11	8	
27	4,000,000	63	33,400		1		2	30	45	20	2	
28							2	25	60	11	2	
30					6			21	49	24		
31					1		1	22	46	22	8	
Aug. 3	3,280,000	55	17,200					13	56	31		Incision and drainage of hip.
5	2,900,000	50					6	5	68	21		
9			13,400		1			17	58	18		
10	2,480,000	50			1			15	50	30	4	
16			17,000		5		2	4	62	26	1	
23	3,120,000	60						4	69	26	1	Purulent glands incised.
			19,500									
Sept. 6					1			2	63	31	3	

CASE 6.—Mr. G., aged fifty-six years was admitted with the complaint of swelling, tenderness, and pain of the right arm. A small incision was made which yielded some thin serous fluid. At this time a deeper incision was not made because the possibility of erysipelas had not been ruled out. Table VI shows very beautifully the inadequacy of the first incision. Instead of the immature neutrophils returning to normal they continued to mount up to 35 per cent. With a revision of the wound and the evacuation of a very large pocket of pus the differential immediately became normal.

TABLE VI

CELLULITIS OF THE RIGHT ARM (CASE 6). SHOWING HIGH LEVEL OF THE STAFFS UNTIL A LARGE PUS POCKET WAS EVACUATED. WITH FREE DRAINAGE THE DIFFERENTIAL COUNT RAPIDLY BECAME NORMAL

DATE	R.B.C.	HB.	W.B.C.	B.	E.	M.	MT.	ST.	S.	LY.	MO.	
Aug. 31	3,200,000	60	5,400				3	12	82	3		Incision and drainage
Sept. 2	3,060,000	55	9,400				3	14	75	8	6	
3								18	60	16		
4								15	61	24		
5					2		3	32	51	7	5	
6								35	53	12		Revision of wound
8					1		2	23	52	10	12	
9								6	64	20		
11					1		1	9	70	15	4	
14					1			6	74	14	5	
15					1			3	65	30	1	

CASE 7.—Miss C., aged four years gave a history of sore throat and abdominal pain. For some time she was treated for tonsillitis. Abdominal symptoms becoming aggravated the patient was sent into the hospital with the diagnosis of acute surgical abdomen. Operative findings showed a perforated appendix with general peritonitis. Table VII shows a leucopenia with an exceptionally severe bone marrow stimulation. Neutrophils showed marked degeneration. Eleven days after operation, however, the staff count was still high. Complication being suspected, revision was performed and a large residual abscess was drained. In this instance we see a postoperative temporary stimulation of the bone marrow with an increase of staff forms. A fecal fistula which developed gradually disappeared. With open-air treatment and forced feeding, the resistance of the patient gradually increased, and the patient, after a prolonged convalescence was cured. With the clinical improvement the staff count gradually returned to normal.

TABLE VII

PERFORATED APPENDIX, PERITONITIS (CASE 7). SHOWING A PROLONGED AND STORMY COURSE WITH EVENTUAL RECOVERY

DATE	R.B.C.	HB.	W.B.C.	B.	E.	M.	MT.	ST.	S.	LY.	MO.	
Aug. 25	3,450,000	60	5,800			1	2	35	40	17	5	Neutrophils degenerated
26	3,780,000	65	5,900				3	37	45	12	3	
27	3,720,000	65	6,600				3	35	37	23	2	
28	3,450,000	65	7,800				1	37	30	21	3	
30	3,490,000	60	11,100				2	27	40	30	1	
31							2	19	48	40		
Sept. 1			13,300				4	32	40	34		
2			18,800					23	48	29		
3			14,000				2	25	50	23		
4								20	55	21	4	Drainage of residual abscess
6						2	5	33	49	11		
7							1	35	37	23	4	
8	3,050,000	60	8,400					20	38	42		
10	•		9,000		2		2	26	34	31	5	
12			9,200	2				31	30	33	4	
14			6,200				2	20	42	28	8	
15						1		14	38	45	2	
21				2				7	41	43	7	

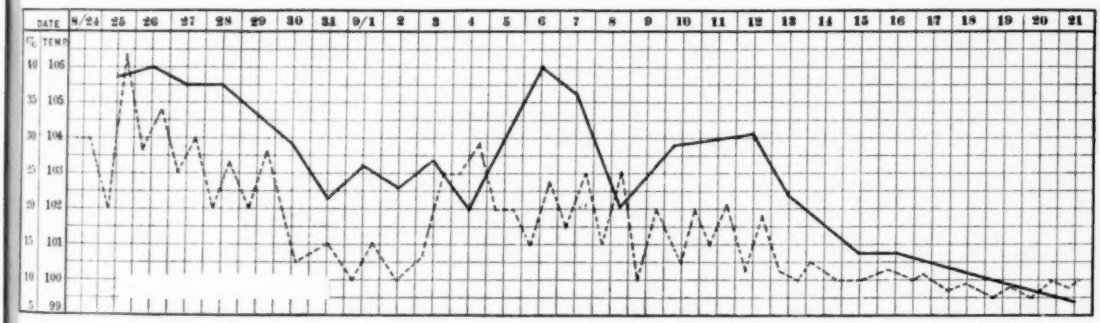

Chart 4.—Case 7. Shows a rise of the temperature and staff curves after the revision for a residual abscess. After this rise the staffs gradually returned to normal.

CASE 8.—Mrs. M., aged thirty-eight years was admitted complaining of severe abdominal cramps, weakness, and anorexia. Four days prior to admission she had had some epigastric pains after her usual supper. Continuation of this pain caused her to sleep poorly. The next morning shortly before rising she was seized with severe abdominal cramps. Eating neither breakfast nor lunch she attended some out-patient clinic. On her way home she was seized with an attack of very severe generalized abdominal cramps and barely managed to reach her apartment. Thereafter she vomited and had considerable gaseous eructations. These cramps continued irregularly for the next three days. Slight relief was obtained by enemas which yielded moderate fecal return. Nausea, retching, and gaseous eructation continued. The patient had had no bowel movements for three days before the onset of her illness. She had not noticed any blood in the stools; neither did she have any chills or jaundice. Because of increasing anorexia and weakness, hospitalization became imperative. Her diet had consisted for the past many years of vegetables and dairy products. The bowels were always sluggish making purgatives frequently necessary. Physical examination revealed a poorly nourished, anemic dehydrated white female apparently very acutely ill. Chest examination showed some dullness and diminished breath and voice sounds over the right base in the axillary region. The abdomen was round and distended. Palpation revealed slight generalized rigidity, probably somewhat more marked in the right upper quadrant. Tenderness was generalized. In the right upper quadrant there was a resistance suggestive

of an indefinite mass. Abdominal reflexes were not elicited. X-ray of the chest showed no infiltration or consolidation of either lung, no pleural changes, no deviation of mediastinal contents. On admission the temperature was 101.6°, pulse rate 112, respirations 30. During the patient's illness her temperature gradually rose to 104.5°, pulse to 150, and respirations to 56. Urine showed 2-plus albumin, few granular casts, a moderate number of white blood cells and few red blood cells. Blood cultures were repeatedly negative; sedimentation rates as high as 68 per cent. Table VIII shows a moderate anemia, a marked leucocytosis with a very marked increase of neutrophils and staffs. There is a concomitant decrease in lymphocytes and monocytes. Chart 5 shows a high constant level of neutrophils and staffs. This case is of additional interest because the consulting surgeon on repeated examination believed that no surgical condition existed. The medical men however because of the suggestive physical findings supported by the laboratory data of a high sedimentation rate and repeated blood examinations indicating pus somewhere in the abdomen, were of the opinion that a surgical condition existed in the environs of the liver. Autopsy showed a subhepatic abscess. In this case the clinical picture aided by the presence of a high staff count that showed no tendency to return to normal should have been sufficient indication for laparotomy.

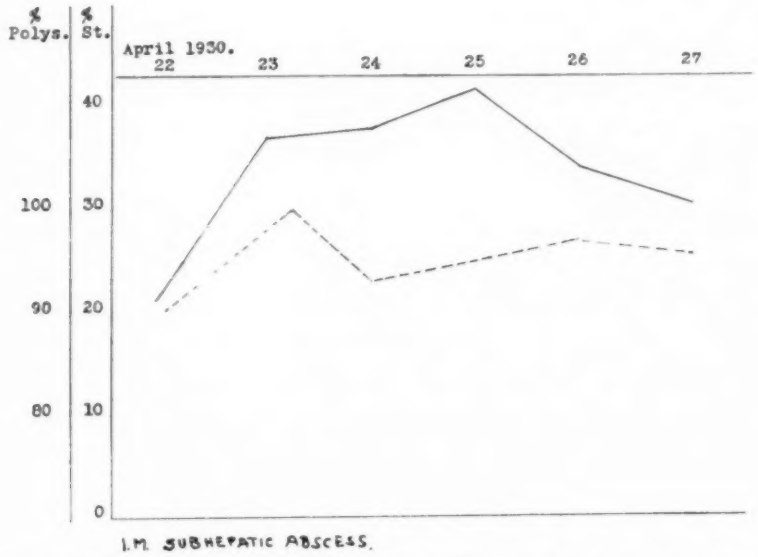

Chart 5.—Case 8. Shows a sustained high level of neutrophils with a higher and more pronounced staff curve. Tendency to rise and to remain up indicates a poor diagnosis.

CASE 9.—Mr. S., aged fifty was admitted with the complaint of pain in the left lower abdomen of twelve days' duration. Twelve days before admission he had suddenly experienced a sharp shooting pain in the left lower quadrant which occasionally radiated across to the right side. This pain was not related to the ingestion of food, exertion or posture, and except for short periods was continually present. The temperature during this time ranged between 101° and 103° F. The abdomen usually became distended in its lower half. Towards the later end of this period he had some pain on defecation. Eight days before admission he collapsed following an enema. Physical examination revealed a well developed well nourished obese male who did not look acutely ill. The temperature was 99° F., pulse 92, respirations 22 and blood pressure 116/80. The abdomen was markedly distended, rigid and tender. This was marked over the left lower quadrant. There was a large mass palpable extending left of the midline, from 5 cm. above the umbilicus to the symphysis pubis. Colon x-ray showed spacticity of the sigmoid and descending colon with intermittent filling. Uranalysis showed sp. gr. 1024, acetone 3-plus, few red blood cells, occasional leucocytes, hyaline and finely granular casts.

TABLE VIII

DATE	BLOOD														
	ERYTHROCYTES NO. IN C.MM.	HB. PER CENT	INDEX	LEUCOCYTES NO. IN C.MM.	TOTAL PER CENT		POLYMORPHONUCLEAR					MONONUCLEAR			PLATELETS	ABNORMAL FORMS
					POLY.	MONO.	MET.	STAF.	SEG.	BAS.	EOS.	L.	S.	MON.		
4/22				25,500	90	10		21	69				8	2		
23	3,920,000	72		17,600	98	2	2	34	62				1	1		
24	3,600,000	71		15,800	93	7		38	55				6	1		
25	3,420,000	71		20,800	95	5	2	40	53				4	1		
26				21,500	97	3	2	32	62				3			1 myelocyte
28	3,470,000	70		28,600	94	6	1	30	61				5	1		2 myelocyte, toxic Degeneration of the neutrophiles

DATE	R.B.C.	HB.	W.B.C.	MT.	ST.	S.	B.	E.	LY.	MO.	MY.	
12/13			65,000	6	25	57	1	0	2	1	8	Toxic degeneration of neutrophils
12/14	5,31	91	66,200	5	31	53	2	1	4		4	Marked anisocytosis

Operation on December 14 revealed a very large perisigmoid abscess. The sigmoid and the small intestine were matted together and adherent to the peritoneum. It is of interest to note that though the blood picture was one of an overwhelming infection, the clinical appearance and well-being of the patient gave absolutely no indication of the gravity of the situation.

CASE 10.—Mrs. K., aged sixty-one years, was admitted with complaints of abdominal pain and vomiting. The onset was sudden with sharp excruciating pain in the right upper quadrant. Pain was knife-like in character and radiated to the back. A few hours later she vomited some greenish fluid. A physician was called in and found the patient's temperature to be 101.2°, and prescribed for the pain. Three days later, the day before admission, she had another attack and was removed to the hospital. Physical examination revealed an acutely ill female who appeared somewhat icteric. The lungs showed a few scattered râles at both bases. There was marked tenderness and rigidity in the right upper quadrant. The temperature on admission was 104°, pulse 112, respiration 24. Chest x-ray showed elevation of the right side of the diaphragm, though freely movable. Uranalysis showed 4-plus albumin, many white cells and granular casts; bile positive. The surgical consultant advised observation.

R.B.C.	HB.	W.B.C.	MT.	ST.	S.	LY.	MO.	
4,340,000	80	13,000	6	27	47	14	6	Marked toxic degeneration of neutrophils

Operative findings the next day were: large amount of bile in peritoneal cavity, ruptured gall bladder with stone in the gall bladder and cystic duct.

With a temperature of 104° F., right upper quadrant signs and a staff count of 33 per cent immediate operative intervention was indicated.

COMMENT

Careful studies of the findings of numerous blood examinations have proved that the total leucocyte count is at best a very coarse indicator of the progress of any infection. It is true that as a rule the leucocyte count will gradually drop as the infection recedes and will remain at a high level, or gradually rise if the disease process is aggravated. Because of its slowness to return to normalcy, an increased leucocyte count may often be found some time after an infection has disappeared. Thus we usually look at the leucocyte count only to inform ourselves as to whether we are dealing with a hyperleucocytosis, a normocytosis or a leucopenia. Very often one or two leucocyte counts are sufficient with the aid of daily differential count to follow an infection.

The second point of the blood examination that always seemed to carry weight was the percentage of neutrophils. The arguments used against the value of the total leucocyte count can also be used here. Generally a high percentage of neutrophils can be interpreted to mean a severe infection and a lower number to signify a milder one. However we have all found patients with 90 per cent neutrophils who were not as sick as patients with 80 per cent. The actual significance of the percentage of neutrophils lies in one of its component parts, the percentage of staff or immature neutrophils. Once this is understood all of the apparent discrepancies can be solved. If a patient has

90 per cent or more neutrophils with but 5 to 8 per cent staffs we can see that the patient has an infection that the body has well in hand. The bone marrow is stimulated and is producing upon demand even more neutrophils than are needed. We are dealing, according to the classification, with a mild form of infection. We can also conceive how, the percentage of neutrophils remaining constant, the severity of an infection can increase proportionately as the percentage of staffs increases in this total number.

We can therefore understand the possibility of having two patients with 90 per cent neutrophils, one desperately sick and the other mildly indisposed. Following the course of the daily blood counts one will often find instances similar to the following: In Table I, on January 11 the neutrophils were 92 per cent of the differential count, on the fourteenth, 95 per cent, and on the fifteenth, 91 per cent. In spite of the fact that all three were above 90 per cent, we know that the count of the eleventh of January was taken shortly before the crisis, and that of the fifteenth the day after. Although the percentage of neutrophils did not show the actual turn of events, a careful perusal of the differential shows a very marked increase of the staff neutrophils, indicating a very severe infection (57 per cent). As the crisis approaches the staffs rise to 70 per cent. The next day the staffs have dropped to 27 per cent. With the victory of the body over its invader the staffs drop either precipitately or gradually depending on the amount of damage caused during the neutrophilic or combat phase.

If the disease process continues to become worse and there is no possibility of removing the purulent focus, the staffs remain the dominant factor among the leucocytes. In these cases the prognosis is poor. To those who employ this classification it must be emphasized that the height of the staff count does not in itself determine the prognosis. Just as in Case 1 where the staff count rose to 70 per cent, the immature cells will often rise to very high figures immediately before a crisis or the removal of an infectious focus. Immediately thereafter they will rapidly drop. On the other hand a constant level of staffs around 20 per cent without any possibility of eradication of the focus will finally be fatal. We found this to be true in Cases 2, 3, and 8. In all three the staff count varied around 20 per cent, finally rising somewhat before death. In Cases 8, 9, and 10 we have three conditions with high staff counts, in which removal of the infectious focus was either not attempted or unsuccessful. Death ensued very rapidly in all three cases.

As a general rule it can be stated that a staff count that rises as high as 50 per cent indicates a fatal prognosis. In patients who recover one usually sees the number of staffs sink to 3 or 5 per cent while the lymphocytes gradually creep from 2 per cent to figures as high as 50 to 60 per cent. This lymphocytic stage indicates the final or healing phase of the infection. We have not discussed the appearance or place of the eosinophil in the course of an infection. Of the actual function of this granular cell very little is known. It disappears suddenly from the circulation, with the onset of an infection and reappears with any evidence of healing. Thus it can be found in subacute and chronic conditions.

SUMMARY

The ten cases here described have been chosen from a large amount of material because they depict the blood picture in some of the most common medical and surgical conditions found. From them we can gather that the actual number of the leucocytes and the percentage of polymorphonuclear leucocytes cannot be used as an indicator of the severity of an infection, nor of the progress and eventual course of the disease. With the onset of an acute infection there is a toxic stimulation of the bone marrow which results in a leucocytosis. This increase in leucocytes is composed mainly of neutrophils. The severity of the infection demonstrates itself by the number of immature neutrophils or staffs.

Table IX gives a classification of infections dividing them into four classes, depending on the percentage of staff neutrophils. Although a single high staff

TABLE IX

CLASSIFICATION OF INFECTIONS—USING PERCENTAGE OF ST. AS INDICATOR

	W.B.C.	NEUTROPHILS				B. PER CENT	E. PER CENT	LYM. PER CENT	MO. PER CENT	
		M.	MT.	ST. PER CENT	SEG. PER CENT					
Normal	5-9,000			3-5	60-70	0.5-1	2-5	20-30	4-8	
1. Mild	9,000		1	5	68			20	6	
2. Moderate	10,200		2	10	67			20	1	
3. Severe	28,400		1	33	50			15	3	
4. Very severe	36,400			41	47			10	2	D
	13,000			79	17			4		D
	4,000			59	17			24		D

count may not indicate a fatal termination, a constant high level of immature cells without the possibility of removing the causative agent is incompatible with life. It will be found that the peak of the staff curve will coincide with the height of the infection. When the staffs remain high after the infection is supposed to have run its course some complication must be suspected. The presence of an increased percentage of the three leucocytic components, even though the number of neutrophils is not increased, indicates a chronic infection. The staff count if taken daily will give more information than the temperature chart, and often even the clinical picture of the patient. Following the neutrophilic or combat phase comes the very short monocytic or conquest phase. Even while this period is still active the final or lymphocytic phase starts. That all of these phases are active can be seen by the presence of signs of hyperactivity of the bone marrow as demonstrated by the neutrophilia and increase in staffs, of the reticuloendothelial system by monocytes showing phagocytosis and of the lymphatic system by the presence of mitoses, Reeder forms, and cells with double nuclei.

To gather the greatest amount of information from the blood examination, serial studies must be made. Where daily differential counts are examined it is not necessary to do daily leucocyte counts. The plotting of a graph showing the daily staff count will usually clearly demonstrate the course of an infection. Rarely does it happen that the staff count is high and the clinical find-

ings of the patient do not agree. Lastly it must be emphasized that the blood picture, though of inestimable value in infections, cannot be used as a differential diagnostic aid to determine the type of an infection. Although negative blood findings cannot supersede positive clinical signs, it must be borne in mind that positive hematologic findings are always of more importance than the absence of physical signs.

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ON THE USE OF LOW-TITERED UNIVERSAL DONORS FOR PATIENTS OF OTHER BLOOD GROUPS, AND SIMILAR SUBSTITUTIONS*

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THE transfusion of blood is frequently an emergency operation, the success of which is dependent, first of all, upon the obtaining of a suitable donor. If the patient is one of the two less common groups, it sometimes happens that a donor of that group cannot immediately be found. Occasionally the urgency is so pressing that the surgeon may consider it advisable to send for a donor without taking the time required for the grouping of the patient.

The emergencies just cited have been met in the past with the use of the universal donor on account of the inagglutinability of the blood corpuscles of this group. The results of such substitutions have usually been satisfactory and Ashby[1] has reported that the Group O (universal donor) corpuscles persist in the circulation of the other groups for as long as six weeks.

The constitution of the blood of the four Landsteiner blood groups with respect to isoagglutination is shown in Table I.

Table I

Serum	Anti-A Anti-B	Anti-B	Anti-A	———
Corpuscles	O	A	B	AB

It is seen that when Group O blood is transfused into a recipient of any of the other groups, there is a theoretical possibility that the transfused isoagglutinins Anti-A and Anti-B will clump the corresponding corpuscles of the recipient; and the actuality of this hypothetical danger would seem to depend on the ability of the transfused O serum to agglutinate the much greater volume of the recipient's corpuscles. This question was first investigated quantitatively by Levine and Mabee[2] who showed that in some Group O donors, the titer of the isoagglutinins was great enough to permit a clumping of ten to twenty volumes of the blood of Group A or AB. This finding was confirmed by Whitehouse and Freeman[3] and also in my own experience. Levine and Mabee referred to the high-titered Group O individuals as "dangerous universal donors." They detected the high titer of the Group O individual in the routine use of Coca's quantitative compatibility test which was designed originally as a convenient direct test of compatibility of the bloods of donor and recipient, but which is also well suited to the detection of donors with high-titered agglutinins.

*From the New York Hospital, the Blood Transfusion Betterment Association of New York City, and Cornell University Medical College.

Coca's compatibility test,[4, 5] which was first published in 1918, has been modified several times by him and his students. The principle of the test is the mixing of the *whole blood*, citrated or defibrinated, of recipient and donor in the ratio that will obtain at the end of the transfusion. In its earlier form, the test was made on the presumption that a patient weighing about 150 pounds and possessing therefore 5000 c.c. of blood was to receive 500 c.c. of blood, that is, a ratio of 1-10. However, in view of the fact that larger quantities of blood are frequently transfused, the ratio was changed to 1-5.

This test is carried out as follows: defibrinated patient's blood is drawn into a white cell-counting pipette* up to the 0.5 mark; the tip of the pipette is wiped off and saline solution is drawn in until the column of blood reaches the 1.0 mark, that is, *ten divisions* of the graduated stem. Saline and blood are expelled upon one end of a slide (avoid bubbles) and mixed with the tip of the pipette. After the pipette is thoroughly rinsed with saline, a similar mixture of donor's blood is deposited upon the other end of the slide. *Two divisions* of this thoroughly mixed 50 per cent donor's blood are carried over with the pipette to the drop of ten divisions of 50 per cent patient's blood and well mixed with it, and this mixture is finally covered with a cover slip and observed for fifteen minutes with occasional slight movement of the cover slip.

The test is a double one. It reveals not only any agglutination of the donor's cells by the recipient's serum, but also any clumping of the patient's corpuscles by the donor's serum in the stated ratio of 5 of cells to 1 of serum.

It is seen in the compatibility test that the defibrinated whole bloods of donor and recipient are mixed in a ratio of 1-5 respectively with the addition of an equal volume of saline solution. The purpose of this dilution is twofold: (1) it facilitates the examination of the film with the microscope; the smaller clumps might be missed if the blood were examined without dilution; (2) the dilution, in most cases, excludes a mistake due to pseudoagglutination.

When Coca's compatibility test is carried out between the blood of Group O (universal) donors and any normal Group A or Group B blood in the place of a patient's blood, no clumping takes place in over half the cases. However, as has been mentioned above, in a number of cases the concentration of the isoagglutinins in the serum of Group O (more often for Group A cells, but also sometimes for Group B cells) is so great as to present a possible danger of a clumping of recipient's cells by the transfused plasma. On account of this consideration, which is supported by some reported accidents, it seems necessary for the present, to avoid the use of universal donors with high-titered sera for transfusions into patients of other groups.

If, in a particular case, the result of this test is satisfactory, it allows the transfusion of the universal donor blood up to one liter, with the following reservation: since the blood of the low-titered universal donor is tested against *normal* blood of the other groups, his blood may become dangerous if transfused into a very anemic recipient of another group; hence, if the red cell count of the 150 pound Group B patient is 2,500,000, he could be theoretically

*The red cell-counting pipette does not give sufficient volume.

allowed only 500 c.c. of the blood of a Group O (universal) donor which has been found usable by Coca's campatibility test.

In the New York Hospital Group O donors are used in substitution for Group B patients and Group A donors for Group AB patients and this practice has been adopted by the Board of Medical Control of the Blood Transfusion Betterment Association. As intimated above these substitutions are made only when donors of the same group as the patient cannot be found quickly enough in an emergency.

High-titered sera among the Group A donors are excluded in the same way as are those among the universal donors, that is, by testing all Group A bloods with Coca's method against a normal Group AB blood.

Since the establishment, in 1928, of the donor bureau of the Transfusion Betterment Association, the suitability of the Group O donors for transfusion into other groups has been routinely examined with Coca's compatibility test; and during the two previous years, the same test was used for the Group O donors of the New York Hospital list.

With the observance of the precaution that we have just discussed, the substitutions have been made in 129 instances, and the universal donor has been used in 29 additional instances in which the grouping of the patient had not been made. No untoward reactions occurred in any of these cases. As it happened, none of these transfusions was followed even by a chill such as occasionally occurs after transfusions of blood from donors of the same group as the patient. The substitutions were distributed as shown in Table II.

TABLE II

DONOR GROUP		RECIPIENT GROUP	NUMBER USED
O	to	A	20
O	to	B	71
O	to	AB	6
O	to	Unknown	29
A	to	AB	32
Total			158

In practically all cases in which such substitutions have to be made, the transfusion is urgently needed on account of hemorrhage; in other words, it is less important that the transfused corpuscles shall be ideally adjusted to the recipients blood than it is that the *blood volume* be restored to a physiologically tolerable point. However, as has been mentioned above, the universal donor corpuscles persist in the circulation of other groups, according to Ashby, for six weeks and, moreover, it still remains to be investigated whether the corpuscles of donors of the same group as the patient last longer than this.

The use of a universal donor for other groups has been recommended by previous observers; in recent years by Blain[6] and by Brines.[7]

Actually we know of one hospital in which the grouping of the patients is omitted and Group O donors are used exclusively. The advisability of such a practice seems doubtful; in the first place because the use of blood

of the same group as that of the patient would seem the most reasonable and unobjectionable, and second, because it would eliminate more than half of available donors. There is a further objection to the indiscriminate use of Group O donors in the instances in which a patient must be given several large transfusions repeated within a short period of time. If the patient is not of Group O, there might result a summation of agglutinative effect by the transfused serum. However, such a theoretic danger could easily be controlled with Coca's compatibility test. Further study in this direction seems indicated.

In one instance in which a physician asked for a universal donor for an anemic person of another group, the physician was advised that according to the result of the quantitative compatibility test, the transfusion of the desired amount of blood (500 c.c.) would cause a distinct clumping of the recipient's blood. Nevertheless, the physician assumed personal responsibility for the outcome and the transfusion was performed without any symptoms whatever.

In explanation of the absence of symptoms one may consider two possibilities; either that a moderate intravascular agglutination need not produce symptoms, which is certainly the case in some lower animals, or that intravascular agglutination was prevented on account of the absorption of the transfused isoagglutinins by the endothelium and other tissues of the body as well as by the patient's corpuscles.*

This experience would indicate that even some of the universal donors with higher-titered sera need not be dangerous for recipients of other groups. Nevertheless, one should hesitate to take the risk of transfusing 500 c.c. or more of universal donor blood capable of clumping from 20 to 80 volumes of the recipient's blood, as in one case tested by me.

SUMMARY

1. A report is made of the successful use of the universal donor for patients of other groups, and of Group A donors for patients of Group AB, in 137 instances.

2. In selecting the donors for such substitutions, those with high-titered agglutinating serum were excluded with the use of Coca's compatibility test.

3. Under the precautions described, these substitutions are recommended when it is not possible quickly to obtain a donor of the same group as the patient.

4. In agreement with the great majority opinion among surgeons and serologists, the use of blood donors of the same group as the patient's seems preferable.

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*It has been shown by a number of observers [8, 9, 10, 11, 12, 13] that the agglutinable substances A and B are not only present in the corpuscles but also in the tissue cells of the body, skin, spleen, kidney, testicle, brain and other organs.

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CERTAIN BACTERIOLOGIC PROBLEMS OF SURGERY*†

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WHAT we know as modern surgery has been developed in the last five decades within the lifetime of many of us and within the professional life of some of those who are at the head of our profession today. This recent development in the art of surgery has been coincident with and has been made possible by the development of the science of bacteriology. Up to the time of Pasteur and Lister, surgery was limited to the treatment of fractures and dislocations, the opening of abscesses, occasional amputations, the removal of superficial tumors and certain emergency measures. Surgery was an external form of treatment and distinct from internal medicine. More than three hundred years ago the Chinese differentiated medicine from surgery by calling the one internal treatment and the other external treatment and these terms are used today. The interior of the body was only approached by the surgeon under the stress of dire necessity because of almost inevitable infection which, if not fatal, subjected the patient to prolonged suffering. Thus both patient and doctor looked forward to any operation with a well-grounded apprehension. The discovery of anesthetics permitted surgeons to operate more slowly and more carefully. They mercifully spared the patient immeasurable torture. They somewhat broadened the scope of surgery but the surgeons' hands were still tied by the threat of infection.

When Pasteur demonstrated that this infection was due to microorganisms and when Lister showed that under certain conditions these microorganisms could be destroyed and infection prevented, even though it took two decades of repeated demonstration for these revolutionary truths to be accepted, surgeons gradually found that they could enter the cavities of the body with impunity and the fear of infection both on the part of the patient and the surgeon gradually disappeared. Then it became possible for regions to be approached which had been forbidden ground and the field of surgery broadened to a degree unbelievable before that time. Now the meninges, the pleura, the pericardium and the heart, the peritoneum, the joints, and the bones may be entered without any evidence of infection. We may now truly say that surgery has ceased to be merely an external form of treatment. Now we may speak of internal surgery.

On the basis of the work of Pasteur and Lister we have built up an elaborate procedure which we call sterile technic. We have developed in ourselves and in our nurses a "sterile sense" which if it is keen, makes us immediately aware of breaks in that technic so that if they occur during an operation they may be corrected at once. But, generally speaking, how keen

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is that sterile sense and how many surgeons the country over are bacteriologically minded? How many wound infections, caused by bacteria introduced into the wound at the time of operation, still occur? In how many hospitals is a close scrutiny made of these infections so that the surgical staff may know whether they occur in 20 per cent or $\frac{1}{20}$ of one per cent of the clean cases operated upon? It is conceded that bacteria fall from the air into practically every operative wound. Still more are introduced by sponges, instruments and hands upon which bacteria have fallen or have remained in spite of the process of "sterilization." Others come from the surface or the deeper layers of the patients' skin. In a certain proportion of these cases infections occur depending upon a variety of factors, the number and virulence of the bacteria, the number and irritating quality of such other foreign bodies as suture material, the trauma to the tissues, the hemostasis and such factors of general resistance as are inherent in the patient, etc. Are these infections beyond our control? Have we reached the irreducible minimum? Such infections not infrequently cause death and if death does not occur the patient is kept in the hospital about twice as long as he would have stayed, if his wound had remained clean. He is subjected to the possibility of herniation in the wound or a recurrence if the primary operation is for hernia. This results in an economic loss to himself and the hospital amounting in the aggregate to a considerable sum of money. The first and foremost bacteriologic problem of surgery, therefore, is the maintenance and improvement of so-called "sterile technique." Surgeons must become bacteriologically minded so that their sterile sense will be keen. We must be on the alert to maintain sterile technic on the highest level that we know. We must discover ways and means for reducing still further the incidence of operative wound infections. Let me be more specific with regard to certain problems in this field. A few years ago our surgical pathologist asked me what was the use of taking cultures from wound infections, inasmuch as the reports usually indicate the presence of one of three or four common species of organisms. I replied that such cultures might give the clue to the source of these infections. Shortly after that time a series of postoperative hemolytic streptococcus wound infections occurred in the hospital. In our search for the source of these infections we cultured among other things the noses and throats of the operative personnel. To our surprise we found that 33 per cent of our staff were carrying this organism in the throat and a number carried it in both nose and throat. At that time surgeons and nurses were masking the mouth but not the nose. Just after this had been discovered another virulent hemolytic streptococcus wound infection occurred in a patient who had been operated upon for hernia, and it so happened that the patient himself had hemolytic streptococcus in both nose and throat. Two of the surgeons operating upon this patient carried this organism in their throats and it was recovered from both nose and throat of the instrument nurse. Using the strain cultured from the infected wound as the key strain, it was now possible by accurate serologic and cultural tests to show that the wound strain was identical with the strains from the nose and throat of the instrument nurse and dissimilar to the strains from the nose and throat of

the patient himself and the strains from the throats of the operating surgeons.[1] Since that time it has been repeatedly demonstrated that these wound infections often occur when a certain individual surgeon or nurse, or a group of surgeons or nurses are carrying these organisms in nose or throat. Now adequate masking of both nose and mouth is a routine requirement in our hospital, not only for surgeons and nurses who perform or assist at operations but for anesthetists and all unsterile assistants in the room. Where recognition of this work has been made this is considered an important feature of the operating room technic, but as one goes about from place to place it is observed that many surgeons have taken no notice of this study and have failed to adopt the necessary precaution. Another specific problem in this field is the question of skin sterilization. In most clinics the tincture of iodine has been used for years but Tinker[2] and others have questioned its efficiency and a number of other chemical substances have been reported to be superior, namely, mercurochrome, acriflavine, picric acid, metaphen, etc. The fact that there is no unanimity of opinion indicates that the ideal skin antiseptic has not yet been found and search must be continued in this direction. Now what of the sterility of catgut? A few years ago a series of postoperative gas-gangrene wound infections occurred in two different hospitals in New York City and in each case it was demonstrated that specimens of catgut, from the same batches as those which had been used in these operations, contained the anaerobic spore-forming organisms of gas-gangrene. This led to a study in our laboratory of the methods of sterilization and the methods for testing the sterility of the final product before its presentation for sale. It was found that the methods of sterilizing, used by the different catgut firms, were all different and some were secret processes. Likewise there was no standard method of testing for sterility. Furthermore it was found that there was no authority requiring the demonstration by the catgut firms of the sterility of their product. All that the surgeons or hospitals had to depend upon was the assurance of the catgut firms whose products they were using, that the material was sterile. But we know that unsterile catgut is being sold by some firms and used by surgeons in their operations at the present time. Specimens of catgut either from their stock supply or purchased in the neighborhood were sent us from twelve different surgical clinics in eleven different states of the Union scattered through the north, east, south and west. There were 174 specimens in all. They were labeled with the names of seventeen different manufacturers or distributers and there were eight unlabeled tubes. The specimens from ten firms yielded no growth while those from the other seven and the unlabeled tubes yielded growth varying from 5 to 67 per cent and of the total number 12½ per cent were found to be contaminated. The contaminating organisms were not the ordinary pyogenic cocci but spore-forming organisms including the organisms of gas-gangrene. The tests for sterility used by a number of catgut firms were found to be inadequate to reveal the presence of anaerobic sporeformers. The problem lay in devising a practical test which would reveal their presence even in small numbers and require its

adoption as a standard test by the catgut firms. Such a test has recently been worked out and it now only remains for the Hospital Standardization Committee of the American College of Surgeons, which has sponsored the study, to agree to require its adoption of all catgut firms whose products they recommend to the hospitals of the country. The four largest catgut firms in the country who have contributed to the financial support of this study have already adopted it and the other honest catgut firms will doubtless fall into line. We hope that this will result in the elimination of unsterile catgut from the market and the elimination from the field of those catgut firms which are either unable or unwilling to put out a sterile product.[3] There is another feature of operative wound infections awaiting serious study, namely, contamination of the field by air organisms. It is probably true that most of the severe infections result from the fairly immediate transfer of pathogenic organisms from one human being to another but many of the milder infections which result in poorly healing wounds and economic loss, are almost certainly due to organisms falling upon the operative field from the air or wafted by air currents from the floor. Lister at first considered these to be of major importance and devised his carbolic spray to minimize this source of infection, but Pasteur believed that they were of minor importance and later Lister came to the conclusion that his elaborate procedures were unnecessary. If a Petri plate is exposed near the operating table, a number of colonies of bacteria will grow out after incubation. This number will vary according to the location of the operating room, its distance from the street, the size of the room, the ventilation, circulation and other air currents, the number of doors and the frequency of their opening, the number of visitors and the cleanliness of their clothing and footwear, etc. As many as two colonies per minute of exposure may develop and this indicates that thousands of these bacteria fall upon the sterile field with every operation. These organisms are chiefly of the staphylococcus group, some yellow, some white; some hemolytic, others nonhemolytic. Occasionally streptococci are found, the hemolytic variety rarely. B. subtilis and molds are frequent, B. coli rare. Unquestionably most of these organisms are destroyed after they reach a wound but all too frequently they result in a trivial or serious wound infection. Just how far hospitals should go in reducing the chance of infection from this source is a question which will have to be worked out in each hospital according to the peculiar conditions present there, but it is a problem of considerable importance and its solution will result in very real monetary saving both by the patient and the hospital.

The bacteriologic problems of surgery that have been referred to above apply to every patient that is operated upon. Inasmuch as our effort is to prevent infections from occurring, we may say that our practice and research here lie in the field of preventive surgery. But our practice also lies in the field of curative surgery and there is an infinite number of problems awaiting solution in this field from the standpoint of infectious disease. In almost every clinic in the country, the department of medicine has been divided into two groups, one caring for and investigating diseases of metabolism and the other caring for and investigating infectious diseases. This has not been

true in our surgical clinics and yet if a census is taken in the surgical wards of a general hospital it will be found that the cases are approximately evenly divided among tumors, infections and injuries or other deformities. If our surgical departments were divided into three groups for the care and investigation of these groups of surgical diseases we would progress farther than we now do with unorganized effort.

In the field of infectious diseases many of our surgical problems are identical with medical problems, for example problems in general resistance against organisms like the pyogenic cocci, streptococcus, staphylococcus and pneumococcus. But the feature of an infectious disease which makes it amenable to surgery is that it tends to localize so that it can be approached surgically and either excised completely like a diseased gall bladder or appendix or incised so that a large part of the infectious material is removed and the balance turned in favor of the patient. Although we often deal with general infections, our chief surgical attack must be local. For this reason it may be more profitable for surgeons in their research in infectious diseases to turn their attention to the local manifestation of immunity rather than to the general. We have reason to believe that recovery from streptococcus and staphylococcus infections is due to the development of a local immunity of the tissues involved rather than to a general immunity. Certainly evidence of the latter often cannot be demonstrated and the failure of passive immunity by the use of sera in these cases helps to substantiate this. On the other hand it has been demonstrated that there is a local immunity, temporary at least, in the area which has recently recovered from a streptococcus and staphylococcus infection and Levaditi[4] has shown that an infected wound from which the infecting organism has disappeared cannot again be infected by the same organism although others may gain a foothold there. This would indicate that this immunity is more or less specific for the infecting organism. Besredka[5] has perhaps gone too far in his theories of "antivirus" but there is enough truth in his ideas of local immunity to open up a large field for research in surgical bacteriology. If in addition to the surgical treatment of localized infections, the defense of the local tissues could be increased materially, the process of resolution might be very much more rapid. This has already been attempted in the injection of staphylococcus filtrates[6] or whole blood in the neighborhood of carbuncles and boils[7] and the use of bacterial filtrates for the dressing of infected wounds but these have hardly exhausted the possibilities of this kind of treatment.

The localization of infection in surgical diseases also suggests the possibility of treatment with bacteriophage. D'Herelle believes that recovery from those infections against which the body is unable to develop a real immunity, is due to the action of a bacteriophage reaching the local lesion via the blood stream and coming from the intestine.[8] While this theory ignores the possibility of local immunity resident in the fixed tissues and wandering cells at the site of the lesion and has not been backed up by proof that such a phage exists either in the exudate from the lesion or in the intestine of the individuals which have recovered, the studies which have been made of the behavior of this interesting phenomenon indicate that there may be a wide field of use-

fulness where there can be made a direct contact between an active phage and the infected surface. D'Herelle claims that it may be injected intravenously and reach the bacteria in an infected focus. Inasmuch as the multiplication of the phage depends upon its association with the organism, it disappears if it does not reach the site of living organisms but if it does reach this site, even in minute quantities, it grows, multiplies and permeates into neighboring areas. The surface has been barely scratched in this field and there is an infinite number of problems awaiting solution.

Intravenous chemotherapy in surgical infections is another important and interesting field. In spite of the vociferous claims of the extraordinary merits of mercurochrome,[9] gentian violet[10] and acriflavine,[11] the wave of popularity for these drugs has passed its peak and they will probably fall into disrepute. The trouble seemed to be that these dyes were too rapidly diluted, too rapidly excreted, and too firmly fixed by certain tissues (which in fact they often injured) for them to reach the focus of infection in potent concentration. Dr. Zau and the writer[12] showed that even if they were injected into the artery supplying the infected part, these dyes never favorably affected the course of an infection with Staphylococcus aureus. And while the failure to accomplish the desired result with these and with other antiseptic dyes has been discouraging, research in this direction should be continued and an effort be made to find an antiseptic which will be nontoxic to tissue cells and have an affinity for pathogenic bacteria.

Another problem of general infection is one which deals with hyposensitivity and hypersensitivity. We have the distressingly frequent development of acute osteomyelitis in children in which there is a septicemia and a localization of the infection in one or more bones without any evidence of reaction to indicate the portal of entry of the organism. Does this point to an absence of a defensive mechanism which might hold the organism for a time at the site of entrance or destroy it there? Is there any way to demonstrate such an absence of defense in children or build it up if it is absent? It is generally believed that the development of an infection at the portal of entry is an indication of sensitivity and the violence of the reaction from the point of view of body defense, indicates the degree of sensitivity. Sensitivity and hypersensitivity have been thought of as stages in the development of immunity and these states must be more fully understood if we are to be able to treat them intelligently. Such local manifestations of hypersensitivity as is represented by hemolytic streptococcus gangrene[13] and such general manifestations as the overwhelming infections which we call "fulminating" and against which at present we are entirely helpless, indicate the importance of a greater knowledge in this field.

Postoperative pneumonia and lung abscess are problems which are as yet unsolved although this field of research has been explored by many workers and a great deal of information has been obtained. Whipple[14] was among the first to point out the prevalence of postoperative pneumonia and was of the opinion that it was generally due to mouth organisms which entered the trachea and bronchi during anesthesia. He emphasized the importance of avoiding elective operations during or immediately after an active cold. He

differentiated this type of postoperative pneumonia, which generally occurs in the first two days after operation from the type which occurs in the second week and which he believed to be embolic in nature. Cutler[15] who studied the problem a little later believed that postoperative pneumonia was due chiefly to infected emboli from the site of operation and pointed out that it occurred frequently in patients operated upon under local anesthesia in which the question of mouth organisms entering the lungs could be ruled out. Lemon[16] demonstrated conclusively by bronchoscopic examination in dogs that when the cough reflex has been subdued by anesthesia the trachea and bronchi contain large quantities of mouth secretion. These secretions are diminished if the cough reflex is preserved or if the head is kept lower than the thorax during the anesthesia. When there has been a tonsil or adenoid operation these secretions may be mixed with blood or bits of tissue. We know how frequently under ordinary conditions food particles or fluids "go down the wrong way" and such nonirritating materials as lipiodol may run down from the larynx into the trachea, bronchi and even bronchioles without producing a cough. It is not unlikely, therefore, that mouth organisms in small numbers frequently enter the lungs and are destroyed. But during operation under anesthesia particularly with the mucus-stimulating ether, these numbers exceed the maximum which can be overcome by the defense mechanism and infection results. W. Pasteur[17] was among the first to call attention to pulmonary atelectasis. Scott[18] and others have more recently described it and have differentiated it from postoperative pneumonia although it is evident that they frequently occur together. The mechanism of this condition seems to be well established. Bronchi or bronchioles become plugged with mucus or closed by spasm. The lung becomes atelectatic and the bacteria in the mucus gain a foothold in the tissues. The demonstration of the respiratory stimulation of carbon dioxide and oxygen by Henderson and Haggard[19] and their use after operation to prevent or treat collapse and pneumonia as advocated by Henderson, Haggard, Coryllos and Birnbaum[20] may result in the reduction of the incidence and severity of this distressing complication but there are still many phases of the problem which require further investigation and elucidation.

Lung abscess presents certain parallel problems. One type which is definitely metastatic by the venous route from an infected focus with peripheral thrombosed veins, must be included in any consideration of septicemia. The other type, the slowly developing, foul smelling, gangrenous variety, in which putrefactive organisms from the mouth play a part, more nearly parallel the postoperative pneumonias for here there is either a secondary infection of a sterile infarct or a direct infection by mouth organisms in the presence of blood or bits of tissue. Although Cutler and his coworkers[21] have studied the pathogenesis of these lesions, the mechanism of their development is still not clearly understood and the prophylactic and active treatment of these patients offer many problems upon which bacteriologic research can throw some light, particularly if it follows the lines of anerobic and symbiotic studies.

Anaerobic and symbiotic bacteriology is also pertinent to the last group of problems that I wish to mention, namely, those revolving about the subjects of peritonitis, and intestinal obstruction. The so-called primary peritonitis, due to the hemolytic streptococcus or pneumococcus, presents a different set of problems from the peritonitis due to disease spreading from the abdominal organs. In the latter group the severe cases are almost always polymicrobic with the principal rôles played by B. coli, B. welchii and the intestinal nonhemolytic streptococci. The rôle of the anaerobes has been a greatly debated question by those who have studied the bacteriology of peritonitis. At first they were ignored because they were not revealed by the methods which were used. Veillon and Zuber[22] considered them to be of paramount importance and they neglected the aerobes. More recent workers in this field have laid varying stress on aerobes and anaerobes. Weinberg[23] felt that the anaerobes were particularly active in the gangrenous lesions of appendicitis. Our own studies have not borne this out.[24] From our recently reported studies in which we attempted to correlate the bacteriologic findings in the peritoneal exudate with the clinical course in over 100 cases of peritonitis we concluded that B. welchii, which we found to be the most common anaerobe present and which occurred in approximately 40 per cent of our cases, was important as a coadjutant with the other organisms, B. coli and nonhemolytic streptococci, but did not give the severe toxicity characteristic of it when it gains a foothold in muscle tissue. We agree with Weinberg in his emphasis on the importance of the synergism of the organisms found in cases of peritonitis, and we believe that no bacterial studies of this disease will be successful which ignore the symbiotic action of the intestinal organisms which are chiefly responsible for the peritoneal inflammation. This is even more important in any bacteriologic consideration of intestinal obstruction. While other factors of a chemical nature play important rôles in this disease, as in peritonitis, the great increase in bacterial content particularly in the upper intestine in obstructed states must be considered and the rôle of the anaerobic bacteria must not be ignored.

Peritonitis offers two sets of problems for bacteriologic and serologic research. The frequency of elective operations upon the intestine, particularly in the removal of tumors from the colon, makes possible the consideration of prophylactic measures to avoid the peritonitis which is the bête noir of intestinal surgery. Although this is a limited field, it offers more immediate results to research than the attempt to control a full-blown peritonitis after it has developed. It has been known for some years that if certain substances are injected into the peritoneal cavity, some defense mechanism is called forth which makes the cavity more resistant to bacteria. Almost any solution that is injected will have some effect but the substances vary considerably in the degree of protection which they give. Saline and even distilled water will give some protection and bacteriologic nutrient broth is still better but the early investigators found that nucleic acid was the best of the substances used.[25] Later, with the idea of using more specific substances, bacterial vaccines were tried. Vaccines from B. coli and green streptococci, as well as nucleic acid have been used by Wilkie[26] for some years

preliminary to resection operations on the gut and more recently Rankin and Bargen[27] have done the same thing based upon the experimental work of Herrmann.[28] But, so far as I know, Herrmann has not demonstrated even in animals that these vaccines give protection against whole intestinal contents and he has completely ignored the anaerobic organisms. The doses which have been used in human beings are very minute (relative to body weight) compared with the doses which have been used in experimental animals. Nevertheless clinical evidence has been brought forward which seems to indicate that some measure of protection has been obtained in a fairly large series of cases.[29] The progress thus far achieved is promising, but we do not feel that there has been sufficient groundwork done to warrant a wide clinical application. This is one of the problems upon which our own laboratory is working at the present time. In the active treatment of acute peritonitis the outlook is more discouraging, but it opens up an infinitely wide and interesting field for bacteriologic and serologic research. The recent attempts at serotherapy with polyvalent anaerobic antitoxin[30] and with antitoxin for B. welchii[31] which at best are weak antitoxins and the "antiserum" for B. coli,[32] the protective value of which has not been conclusively demonstrated, indicate the direction in which research and even clinical thought has been trending but success has not been consistently claimed even based upon clinical results which must always be accepted with caution. It is not inconceivable that the solution may lie in the discovery of a polyvalent bacteriophage potent for all of the important intestinal organisms.

I have briefly touched upon a few of the bacteriologic problems of surgery. There are many more but these will serve to give some idea of their scope, importance, and complexity. Here are problems which might profitably occupy the attention of groups of workers in every clinic of the world. Where shall we find the workers? Is the surgeon too busy in practicing his technic to give time to them? Can these problems be solved by bacteriologists working in departments of bacteriology or attached to departments of surgery? I think rather it can best be done by young men well trained both in surgery and in bacteriology who from the start of their surgical careers will be bacteriologically minded, men who will maintain a bacteriologic viewpoint in all of their clinical work and a clinical viewpoint in all of their bacteriologic work. Can we find such men in every important clinic? I think we can.

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LABORATORY AIDS IN THE DIAGNOSIS AND TREATMENT OF SURGICAL SEPTICEMIA*

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THE term "septicemia" is commonly applied to states associated with positive blood cultures but an increased knowledge due to the perfection and extended use of blood-culture methods, has shown that in many diseases not designated or recognized as septicemia, bacteria may be found in the blood as in pneumonia, typhoid fever, meningococcus meningitis, etc. Indeed it would appear almost impossible to give a definition of surgical septicemia free of all objections, but in this communication is used for designating a group of cases in which the symptoms due to a more or less constant presence in the blood of pyogenic bacteria like streptococci, staphylococci, pneumococci, gonococci, etc., overshadow those of local origin and characterized clinically by septic temperature, chills and sweats. Such may be designated by its source, as puerperal, osteomyelitic, mastoidal, etc.; also by naming the microorganism as well, as "osteomyelitic staphylococcus septicemia," "puerperal streptococcus septicemia," "anthrax septicemia," etc.

Clinical diagnosis may be possible by the symptoms and signs alone but not infrequently depends upon laboratory procedures with special reference to blood cultures. Treatment includes the prompt institution of thorough and adequate drainage wherever possible along with careful nursing, rest, selected diet and adequate fluid intake. But the prompt recognition of the condition, and the prompt institution of a definite method of treatment almost invariably demand certain laboratory aids which I shall endeavor to briefly summarize herewith.

LABORATORY AIDS IN DIAGNOSIS

1. First in importance are *blood cultures*. Indeed septicemia associated with surgical diseases is sometimes first detected by them and the absence of classical signs and symptoms by no means excludes the possibility. As the presence of bacteria in the blood may be intermittent, two or more cultures at intervals of one or two days may be required. As a general rule microorganisms develop rapidly in blood cultures in surgical septicemia but all should be kept under observation for at least five days before reporting sterile results.

Great care is required in taking the blood to guard against contamination with staphylococci (particularly Staphylococcus albus) and streptococci (particularly nonhemolytic types) from the skin. Indeed it is usually ad-

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visable to repeat the culture at least once before arriving at the diagnosis of a Staphylococcus septicemia in order to guard against error due to contamination.

As shown by Ottenberg[1] there is an advantage in taking blood for culture from a vein draining an infected area whenever this is possible. Cultures of blood from the internal jugular veins of individuals with suspected lateral sinus thrombosis in mastoiditis have for example, yielded positive results when cultures from veins at the elbow were sterile or have shown a smaller number of bacteria. In his opinion the finding of a much greater number of bacteria in the blood from one internal jugular vein than in the other confirms the diagnosis of lateral sinus thrombosis although it is impossible from the count alone to tell on which side the thrombosis is located.

As a general rule it is a good practice to culture relatively large amounts of blood like 5 to 10 c.c., and I have found glucose harmone broth with a P_H of about 7.4 to 7.6 quite suitable in 100 to 200 c.c. amounts. By plating measured amounts of blood like 5 c.c. with 10 c.c. of glucose harmone agar, an idea of the numbers of bacteria per cubic centimeter may be obtained which is of value in estimating the gravity of the infection and prognosis as well as being a guide in evaluating the results of treatment.

Unfortunately we do not have available at present a practical method for detecting or measuring the amounts of bacterial toxins which are commonly believed to be present in the blood in septicemia. It is likely that skin tests employing the serum of the patient injected intracutaneously in the lower animals and especially the rabbit, may be of aid in this connection, but mice, rats, guinea pigs, and rabbits possess such a high natural immunity to staphylococci and streptococci, that I, at least, have not been able so far to work out an acceptable method by intraperitoneal and intravenous injections of serum.

2. In this connection I may also mention that it is sometimes of value to *estimate the bactericidal and bacteriostatic activity of the blood* in surgical infections to determine at the outset the patients' chances of developing septicemia. This is done after a method of Cohen[2] consisting in placing a small amount of pus or culture in the bottom of a sterile test tube and adding 5 c.c. of blood. If organisms develop in this whole coagulated blood, it may indicate a breakdown of immunologic resistance and the possibility of the development of a blood stream infection. If organisms do not develop, the reverse may be true although I have found septicemia to develop under such conditions apparently the result of a subsequent reduction in immunologic resistance.

3. *Total and differential leucocyte counts* are of course well known and appreciated in the diagnosis of septicemia. A leucocytosis largely due to an absolute increase of polymorphonuclears along with a relative or absolute decrease of eosinophiles has long been accepted as the typical change.

But an estimation of the metamyelocytes or immature polymorphonuclears greatly improves the value of the differential leucocyte count in diagnosis and also as a means for estimating the severity and progress of septi-

cemia. These cells are readily detected and divided into two types, the young and the old or banded forms. In the usual differential count they have been included in the percentage of large lymphocytes, transitionals and polymorphonuclears and their significance entirely lost. By this newer method, however, it is possible to obtain valuable information even when the percentage of polymorphonuclears is about normal.

Normally the blood contains none of the young metamyelocytes and about 4 per cent of the older ones. In acute infections both are increased and designated by Schilling as a "shift to the left." This shift may occur with but a slight and insignificant increase of the polymorphonuclears. By dividing the number of polymorphonuclears by the number of metamyelocytes, my colleagues, Boerner and Gerard,[3] have worked out the "nuclear index" as a means of reporting. For example, there is normally about 4 metamyelocytes to 60 polymorphonuclears giving an index of 15. If the metamyelocytes increase, the index becomes lower so that an index of 10 to 15 constitutes a slight shift to the left, 5 to 10 a moderate shift, and below 5 a marked shift. *I strongly advise surgeons to adopt this shift to the left and nuclear index method for routine differential leucocyte counts and reports.*

Furthermore, as shown by Boerner[4] it is advisable to abolish the usual custom of reporting the different leucocytes by percentages since it is possible for the polymorphonuclears and other types to be present in a normal percentage while being actually increased. It is much better to report the actual number of each variety per c.mm. of blood, the normal for older children and adults being as follows:

Lymphocytes	1000 to 3000
Monocytes	100 to 600
Polymorphonuclears	3000 to 7000
Eosinophiles	50 to 400
Basophiles	0 to 50

This is a more accurate and useful method since it gives the "absolute" number of the different leucocytes, as one or more types may show important changes which are not revealed by the usual percentage or "relative" system. In surgical infections particular interest is attached to the polymorphonuclears and eosinophiles and I recommend the adoption of this plan by surgeons even though it entails the task of forgetting percentages and training the mind to interpret the results on the basis of the total numbers of the different leucocytes per cubic millimeter of blood.

LABORATORY AIDS IN TREATMENT

1. *When antistreptococcus serum is employed for the treatment of streptococcus septicemia, there may be an advantage in testing several sera for their agglutinating titers for the streptococci secured in cultures* and employing that which shows the highest titer, although the agglutination power of a serum is not an exact measure of its efficacy, nor does the absence of agglutinins necessarily indicate that such a serum is without specific and nonspecific therapeutic value.

2. *As blood transfusions are usually employed in treatment, the very careful matching of the blood of patient and donor is required* in order to avoid transfusion reactions as much as possible.

In this connection it is always advisable to employ direct matching tests. Donors may be selected belonging to the same group as the patient but if time and facilities permit, the final selection of a donor should rest upon direct tests in order to avoid the regrettable error of using a donor of a subgroup. This is particularly true of Group A donors (corresponding to Group II of Moss and Jansky). I also strongly recommend in the interests of safety and simplicity dropping the Moss and Jansky classifications and employing only that of Landsteiner:

Landsteiner Group	A	= corresponding to	II	of Moss and	II	of Jansky.
Landsteiner Group	B	= corresponding to	III	of Moss and	III	of Jansky.
Landsteiner Group	AB	= corresponding to	I	of Moss and	IV	of Jansky.
Landsteiner Group	O	= corresponding to	IV	of Moss and	I	of Jansky.

Without doubt blood transfusion is a valuable part of the treatment of septicemia for the purpose of not only supplying fresh leucocytes for the elaboration of bactericidal substances if the patient's leucocytes are exhausted and incapable of immunologic response but also for the purpose of supplying the patient with complement, as his own is likely to be reduced below normal levels.

But transfusions are of no value as a substitute for surgical drainage because if the blood is being continuously flooded with organisms and toxins from infected tissues, we cannot expect to save or even greatly aid the patient by transfusion or any other method of biologic or chemotherapy.

3. *Since transfusions with the blood of immunized donors is regarded by some as an added advantage, several methods of "immuno-transfusion" have been advocated.*

The term was first used by Sir Almroth Wright[5] for a method consisting of adding a quantity of vaccine, like 1,000,000,000 stock staphylococcus vaccine to about 500 c.c. of defibrinated blood from a compatible donor; after waiting for about an hour the mixture was given the patient intravenously. According to Wright this procedure results in an elaboration of nonspecific bactericidal substances from the leucocytes of the donor's blood effective upon all ordinary pathogenic bacteria and not only upon the organism with which the blood had been vaccinated.

In my experience it was found that this method of "vaccinating blood in vitro" results in an increase of bactericidal activity but intravenous injections were usually followed by rather sharp reactions which I thought were caused by the bacterial protein and that the therapeutic effects were due at least in part to this nonspecific bacterial protein reaction.

Later, Colebrook and Storer[6] suggested a method for determining whether an individual patient ought to be treated by immunotransfusion by testing the phagocytic activities of the leucocytes and if the phagocytic intake is one-third or less than that obtained with a normal blood, to employ immunotransfusion by the following method:

A compatible donor is given a subcutaneous injection of 1000 million stock staphylococcus vaccine and his blood used four or five hours later. Colebrook and Storer advised the use of defibrinated and not citrated blood. I have employed the method with apparent success by using direct methods of transfusion as I observed less reaction in patients with no danger of bacterial contamination of the blood.

Still later Hooker, Dick and others employed donors previously immunized with a vaccine prepared of the infecting organism of the individual patient consisting in general terms of the subcutaneous injection of 500 million, 1000 million and 2000 million heat-killed vaccine on successive days followed by transfusion about one week later.

One drawback to this method is the time required for the immunization of the donor although I have found the method particularly applicable for the treatment of those cases of staphylococcus septicemia surviving the acute stage and requiring transfusions later on. In all instances of staphylococcus septicemia it is my practice, therefore, to secure one or two compatible donors as soon as possible and start their vaccination in order to have them in readiness in from ten to fourteen days if transfusions are required as is generally the case.

4. Since immune sera for Streptococcus septicemia may not contain antibodies for a particular strain and as antistaphylococcus serum may not be available at all, Cadham[7] has sought to develop *a method for preparing autogenous sera and administering these in conjunction with normal serum from compatible donors to furnish the complement deficiency so frequently observed in septicemia.*

The method adopted was as follows: "The invading micro-organism was obtained from blood cultures and grown in serum-glucose broth. The resulting growth having been centrifuged three times in normal saline, a vaccine, in which the micro-organisms had been heat-killed at the minimum lethal temperature, was then prepared, and inoculated into rabbits and guinea pigs. These animals tolerate comparatively large doses of a vaccine containing either streptococci or staphylococci, especially if the organisms are washed free of the media. The dose of the vaccine, starting with one-quarter billion and working up to three billion organisms, was given on alternate days. An agglutination titer of one to five thousand may be obtained as early as the sixth day. Any time after the fifth day blood was drawn from the heart with aseptic precautions and without causing the death of the animal. This blood was placed in the ice chest for eight hours, and then the serum was pipetted off. The patient was inoculated subcutaneously with from three to four c.c. of this serum. Twelve patients were treated with rabbit serum, and six with guinea pig serum. There seems to have been no appreciable difference in the results obtained.

"A donor whose blood was completely compatible with that of the patient was obtained as soon as possible. The donor reported at the laboratory and from 50 to 60 c.c. of blood were withdrawn in large vacuum tubes. This was left at room temperature for fifteen minutes and then placed in the ice chest for fifteen hours. The serum was next pipetted off with all

aseptic precautions, examined for sterility, diluted with equal parts of saline, and given by means of a syringe to the patient intravenously. Thus, the patient received an inoculation of the animal serum containing the antibodies subcutaneously, and also received a transfusion of from 25 to 30 c.c. of the donor's serum containing complement. Originally, the treatments were given one week apart, but this was subsequently shortened to two-day intervals. Treatment was continued until negative blood cultures were obtained and the patient showed considerable improvement. The greatest number of treatments given to any one patient was seven.

"To isolate the infective organism, culture it, inoculate the animal with the vaccine, and to await the development of amboceptors of value, required at least five days; and to obtain a serum with a more powerful agglutination titer, twelve days. Frequently, the emergency of the case did not permit any such delay. To overcome this difficulty some rabbits and guinea pigs were inoculated with various strains of streptococci and staphylococci. Certain animals received inoculations of a vaccine prepared from fifteen strains of streptococci, which had been obtained from as many different cases of local or general sepsis. Patients were treated at once with the serum from these animals, pending the development in other animals of a specific serum."

5. *Skin tests for allergic sensitiveness to the serum of the horse or other of the lower animals* is not strictly a laboratory procedure but may be included herewith since they are usually referred by surgeons to clinical pathologists preliminary to serum therapy and especially by intravenous injection.

The intracutaneous test consisting of the injection of 0.2 c.c. of a 1:10 dilution of serum is usually employed and I generally inject the serum selected for treatment rather than normal horse serum.

A conjunctival test consisting in placing a drop or two of 1:10 dilution of serum into one of the eyes is also employed but at this writing I am not able to express an opinion of their relative sensitiveness and practical value. If either or both yield positive reactions the injection of serum should be given with special precautions but I do not give a horse immune serum at all in any amount or by any route or method to horse asthmatics giving positive skin or eye reactions.

6. If bacteriophage is employed by local application to infected wounds or by injection about the area of infection or by intravenous injection, I believe that *it is essential to first determine in the laboratory if the culture is susceptible to lysis by the bacteriophage to be employed.* This is readily determined in the laboratory and I see no reason for using bacteriophage in treatment unless laboratory tests show that the microorganism of the septic infection is susceptible.

7. *Finally, the laboratory is indispensable for determining the toxicity and probable therapeutic value of chemical agents advocated for the chemotherapeutic treatment of septicemia.* While this is not a matter of routine laboratory examination, yet there is not the slightest excuse or reason for giving human beings any chemical compound by intravenous or intramuscular injection until an adequate idea of its toxicity has been first obtained by suit-

able animal experiments. And these tests are sufficiently simple to be conducted in any well-organized laboratory.

SUMMARY

1. Laboratory aids in the diagnosis of surgical septicemia: (a) Blood cultures by ordinary and special methods; (b) estimation of the bactericidal and bacteriostatic activity of the whole blood; (c) total and differential leucocyte counts; (d) estimation of the metamyelocytes of the blood, "shift to left" and "nuclear index"; (e) estimation of total numbers of various leucocytes instead of percentages to obtain changes in "absolute" numbers instead of "relative" changes.

2. Laboratory aids in the treatment of surgical septicemia: (a) Selection of antistreptococcus serum on the basis of agglutination tests with the infecting streptococcus; (b) careful selection of donors by group and direct matching tests for blood transfusion; (c) methods for "immuno-transfusion"; (d) method for preparation of autogenous streptococcus or staphylococcus serum and supplying deficient complement of the patient; (e) skin tests for serum allergy in relation to serum therapy; (f) selection of bacteriophage by laboratory tests of lysis for the staphylococcus or streptococcus producing infection; (g) toxicity and therapeutic tests in the lower animals of chemotherapeutic agents employed for the treatment of surgical septicemia.

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BIOPSY IN THE TREATMENT OF MALIGNANCY

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BIOPSY comes from the Greek words *life* and *vision*. It really means the inspection of the living body as opposed to necropsy, a similar examination of a dead body. Every operation, therefore, is a biopsy. Today, however, the word has a very limited meaning—it is confined to the examination of a piece of tissue from the living body removed for microscopic diagnosis.

Today I examined the frozen section of a piece of tissue which a woman noticed in her urine and which apparently came from the vagina. The microscope showed it to be the mucosa of the uterus and the cellular morphology was so changed that it was difficult to be quite certain of the character of the cells in which the glands of the uterine mucosa were imbedded. Such an examination would not be, strictly speaking, a biopsy.

Biopsy and Radiosensitiveness.—Our views in regard to the indication of biopsy are changing and progressing as we accumulate more and more data and as we restudy our old records, especially in relation to the ultimate results. The earlier the patient comes under observation, the more difficult the clinical diagnosis, until we reach a period when all clinical signs and laboratory tests fail. When the lesion which we can see and feel is so small that it can be completely removed without mutilation, there is no necessity for biopsy, except in the sense that, after the tissue is removed, it can be studied in the frozen section at once.

In the strictest sense, biopsy is the excision of a small piece of living tissue, no matter how this small piece is obtained, and its immediate examination in the frozen section. Often, however, this microscopic study is postponed for a permanent section. Nevertheless it is still called biopsy. We are gradually coming to the conclusion that a biopsy should have for its object the examination of a piece of living tissue from an area in the living body when there is no other diagnostic procedure which will reveal its exact nature and indicate the best method of treatment. In recent years many cancer students are thinking of radiosensitiveness of tumors as a possible form of biopsy. There is much evidence that the trial of x-rays or radium should be the procedure of choice in many instances before exploratory incision and the excision of a piece of tissue for frozen-section diagnosis.

For example, the last case I studied this afternoon allows me to discuss this problem. An adult male, about forty-two years of age, observed a lump in the neck below the middle third of the lower jaw and outside and below the digastric triangle. It was not the submaxillary lymphatic gland. There were no other palpable lymph glands in the neck, axilla or groin. There was no palpable enlargement of the liver or spleen. The patient is a dentist and his teeth are in perfect order. Recent x-ray films of the teeth show no root

abscesses. He did not look anemic. There had been no pain. He had experienced no weakness or early fatigue. The patient simply felt accidentally a nodule in his neck. In this instance certain preliminary examinations had been neglected. The Wassermann test had not been made, nor the blood counted for leukemia. The tonsillar and nasopharyngeal area had not been examined. An x-ray of the chest had been made and reported negative, but no x-ray had been made of the neck to ascertain whether the tumor cast a shadow or whether it was calcified. Without making these preoperative tests and without considering the possibility of what the condition might be and without considering the advisability of preoperative radiation, an incision was made in the neck under local anesthesia of sufficient length to have allowed the complete removal of the palpable tumor. Apparently a very small piece of what appeared to be an enlarged gland was removed. The size of the tissue which the patient brought with him in formalin was less than 3 mm. in diameter. The diagnosis from the permanent section made at his home was lymphosarcoma.

We made a section of the tissue and confirmed the diagnosis of lymphosarcoma. An x-ray of the chest was made and there was no evidence of a mediastinal tumor, calcified glands, or any abnormal changes in the lungs. No tumor shadow was seen in the x-ray of the neck and no calcification. The complete examination of nose and throat must be postponed until tomorrow, and although specimens of the patient's blood were taken, we cannot expect a report for at least a day.

It was decided to give this man x-ray treatment at once and not wait for any further results of examinations, because this should have been the method of treatment in the first instance before the biopsy and after the complete examination had been made.

We know that lymphosarcoma and Hodgkin's disease usually begin in the glands of the neck and that in many instances one involved gland is the first symptom of onset and may be present for some time before other glands are involved or other signs are present. If the enlarged gland is due to metastasis and the primary focus cannot be found, removal of the gland will have only diagnostic, not curative value. When the gland is due to a focus of infection, removal of the gland and finding no evidence of metastasis, Hodgkin's disease or lymphosarcoma, will not help in the search for foci of infection.

The point I wish to bring out in discussing this case is that biopsy is not a procedure to rush into at once, although if one gets a piece and makes a good section, the sections can be referred to one or more competent pathologists and their diagnosis received in a relatively short space of time.

I venture to make the statement that the medical advisers who are considering biopsy and are not in a position to make an accurate diagnosis and propose to refer the sections to more competent pathologists, should not perform the biopsy, but should make a thorough physical and clinical examination and all the laboratory tests possible and what x-rays are necessary. Then these data should be referred to a consultant chosen before biopsy is performed.

In the case we have just discussed the preoperative study was incomplete. Had the case been referred to a consultant the advice to complete it would have been received.

The next mistake was that the gland should have been completely removed, even if it was only one of many glands, and especially so as it is the only gland. There is no danger that we have ever ascertained from the removal of a single gland for diagnosis if ultimately it proved to be lymphosarcoma, Hodgkin's disease, tuberculosis, or a chronic lymphadenitis of any type. But it is a mistake and may reduce the chances of a permanent cure to remove a gland when there is a definite primary tumor. The nature of such a primary tumor must first be ascertained, if possible. This, however, needs further discussion. In the case under study now the single palpable gland in the neck should have been removed, because since the biopsy it has increased in size and, if it does not disappear under x-ray treatment, the patient will have another minor operation.

Had we been consulted in this case when the tumor was first observed and before the biopsy as we have been consulted after it, we would have advised deep x-ray therapy first, especially if no focus of infection had been found in teeth, tonsils or in the nasopharynx.

In this case the position of the enlarged gland is evidence against an oral, nasopharyngeal or sinus infection. It is more likely to be the beginning of Hodgkin's or lymphosarcoma. It is not the gland you would expect to be first involved in a cancer of the lip, oral cavity or nasopharynx. It might be the gland first involved in a cancer of the larynx.

I am prolonging this discussion here, because at the present moment it impresses me as most important along with the indication of preoperative radiation. Simpler points in biopsy have been considered in previous publications, references to which will be given later.

The technic of a biopsy is as a rule relatively simple. The decision as to a biopsy, even in the hands of the most experienced, is often very difficult.

In every case, previous to biopsy, the question of irradiation with x-rays or radium first, must always be carefully thought out.

A second observation should be recorded here.

An adult male, of about fifty years, was referred to the clinic because he felt a lump on the left side above the larynx. It had been present about one month. The patient was a physician and recently had been overworked in sedentary pursuits. He was getting little or no exercise and sunshine. There had been a little loss of weight and too early fatigue before the end of the day's work. There were no symptoms on part of the voice, nor a cough to point to the larynx. There was but one suggestive symptom in the history—some ten or more years previously a slight infection of the skin over the left shoulder was associated with the enlargement of the lymph gland now involved above and slightly to the left of the larynx. The lymph gland was no larger than the end of the thumb. It was not tender. The positive findings of a complete examination were: Slightly enlarged tonsils which, when aspirated, contained many minute pockets filled with purulent material in which were found streptococci, pneumococci and staphylococci.

The patient was unaware of any recent sore throat or infection of any kind. This gland is not usually involved in a tonsillar infection. I advised the removal of the tonsils and the immediate irradiation of the involved gland and a wide area of the neck. The gland subsided gradually but completely. The patient improved slowly but steadily. As he was an intelligent physician, he knew the possibilities of Hodgkin's disease and lymphosarcoma.

If we had removed a small piece of this gland as was done in the first case discussed here, it might have been an enlarged gland due to infection in which we were unable to find germinal centers and for this reason would have considered lymphosarcoma. If we had removed the whole gland, there would have been the problem of differentiation between a chronic lymphadenitis and the earliest stage of Hodgkin's and lymphosarcoma. Removing the tonsils was indicated in any event. Irradiation gives the only hope of a cure of lymphosarcoma and Hodgkin's disease. You might say in this case removal of the gland for diagnosis would have added to the scientific interest of the case, but it would not have added to the probability of a cure. On this point there will be difference of opinions, but there can be none on the value of a complete preoperative study, on the removal of foci of infection if found, and on the value of irradiation which can do no harm, and is the only thing we have for lymphosarcoma and Hodgkin's disease.

Some three years ago a gland was received in the laboratory for diagnosis. The patient was a boy of fifteen years. On careful microscopic study we concluded that there was no evidence of lymphosarcoma or Hodgkin's disease. Unfortunately, two years later during which time the boy had been well, a second gland was removed which was the typical picture of lymphosarcoma. The disease is being held up to the present time with x-ray treatments. But two years were lost by this delay.

Under this heading of Biopsy and Radiosensitiveness I have emphasized the great importance of preoperative study, preoperative radiation as a therapeutic test, and when ultimately a consultation will be asked for, the greater value of this consultation, even if it is by letter or telephone, before and not after the biopsy.

Biopsy in Breast Lesions.—This presentation is taken up second, because since my last paper in 1929 further intensive studies in the Surgical Pathological Laboratory of the Johns Hopkins Hospital, and the most careful review of our borderline tumors which have accumulated since 1890, have somewhat changed our views in regard to biopsy in doubtful breast tumors.

At first the evidence indicated an added danger if a malignant tumor of the breast was removed, the wound closed and then, later, after a microscopic diagnosis, the complete operation for cancer was performed. This danger seemed to be present even when the glands in the axilla were found to be uninvolved at the second complete operation. For this reason most surgical pathologists advised the complete operation when the surgeon and pathologists were in doubt as to the malignancy of the breast tumor and, as a matter of fact, this procedure was practiced in most clinics. For example, the tumor is clinically benign; the surgeon explores it; a piece is either cut out for biopsy, or the entire tumor is removed, bisected by the operator and

studied. In either event a frozen section is made. Both the surgeon and the pathologist are in doubt. Until very recently it seemed to be the best advice that the complete operation be performed at once.

My own recent studies which include a restudy of all the tumors of the breast that have been explored of which we have records since 1890, force the conclusion that, if the surgeon has had average experience with breast tumors and the pathologist who interprets the frozen section has about the same qualifications, and when both are in doubt as to malignancy, it is just as safe for the patient to completely remove the tumor and refer the sections to more experienced pathologists.

Of course, this conclusion is based upon facts, but there is a definite explanation. In the first place, the number of well-trained pathologists qualified to make a differential diagnosis of breast tumors is increasing. In the second place, the majority of surgeons with a fair amount of experience with breast tumors can tell cancer in the gross. The moment they are in doubt from the naked-eye appearance the probabilities are that the tumor is benign. The most significant evidence, however, is this—the number of borderline tumors of the breast which are clinically benign and should therefore be explored is rapidly increasing, even in the smaller clinics. This increase is due to the shorter interval between the first discovery of the tumor and the operation. The most common cause of a tumor in the breast of one month's duration is chronic cystic mastitis. As time goes on, the tendency is that this tumor will disappear and it becomes relatively infrequent as the duration of the tumor increases. It is true that when these borderline tumors are completely excised and the wound closed and sections are referred to a number of pathologists, there will be many disagreements, with the result that a number of patients will be subjected to the unnecessary complete operation. But none of them will die of cancer, because the condition is not cancer. Fortunately, more surgeons are interesting themselves in pathology and in more and more operating rooms in the standardized hospitals throughout this country there are pathologists to make and interpret frozen sections. This improvement is largely due to the American College of Surgeons.

I have written a short article on Borderline Breast Tumors for the International Contributions to the Study of Cancer in honor of James Ewing. I am preparing a larger study for the new *American Journal of Cancer*.

Our last experience on borderline tumors is rather significant. The tumor was clinically benign. The surgeon who removed it was doubtful as to its nature. At first he would not agree with the frozen-section diagnosis of his pathologist. Finally he did. When the sections were submitted to a consulting group, all agreed that the diagnosis of the first pathologist as to a benign lesion and chronic cystic mastitis was correct. Nevertheless sections similar to these were submitted by me in 1915 to the most experienced surgical pathologists in this country. The diagnosis of the majority was "malignant." Submitted to a larger group in the Surgical Laboratory of the Johns Hopkins Hospital and University last June and September (1930), the majority voted in favor of benignancy.

Again we see the great change that is taking place in our attitude toward biopsy, and its increasing value is due to the training of the microscopic pathologist who is becoming especially experienced in the study of frozen sections in the operating room. Cancer is becoming a microscopic disease, and the medical profession is rapidly preparing to meet this new demand.

Remember, Velpeau in the first edition of his book on *Diseases of the Breast in 1854*, seventy-seven years ago, wrote the book without any microscopic evidence. In a second edition a few years later he complained of the diagnosis of the microscopic pathologist as usually being incorrect, chiefly in calling benign conditions malignant. In the seventy years since that date, in spite of the great progress in our knowledge of general pathology, there has not been an equal progress in the microscopic interpretation of tumors of the breast, bone, uterine scrapings, etc. In the first place, the pathologists giving special attention to tissue diagnosis in the operating room have been relatively few, and of these those using frozen sections still fewer.

My evidence confirms the expressed opinion of a few observers who have really studied the problem—that for many years a surgeon who is a surgical pathologist made very few mistakes in his gross pathologic diagnosis. Then he began to meet a larger number of borderline tumors which he was compelled to submit to microscopic study. The pathologist did not overlook cancer. No one with any training in microscopic pathology will mistake fully developed malignant disease. But these borderline tumors exhibited microscopic pictures closely simulating malignant disease. In the breast they were apt to be called adenocarcinoma, malignant adenoma, areas suspicious of malignancy, and the breast removed or the complete operation for cancer done. Some of the patients consented, some refused. So that in the end there were three groups—tumor only, breast only, and the complete operation. In the latter group the glands were not involved. Those few clinics which followed their cases through for years first classified them with adenocarcinoma or malignant adenoma of the breast, or cancer in chronic cystic mastitis, or cancer in papillomatous cysts, cancer in chronic mastitis in a lactating breast, sarcoma in intracanalicular myxoma, cancer in encapsulated adenoma, cancer in encapsulated cystic adenoma, suspicious areas of cancer in intracanalicular myxoma. The surprising outcome was that these patients all lived, no matter what the diagnosis, or what the operation was. When followed from five to twenty years, they did not show 10 per cent of cancer in the remaining breast. When they returned with a lesion of the remaining breast, it was either distinctly benign, or similar to the previous tumor. For this reason many of these patients lost both of their breasts. In 1915 I gathered this group together and removed them from cancer of the breast in which the glands were not (microscopically) involved. This withdrawal of the borderline group, reduced the five-year cures from 85 to 70 per cent.

As I propose to discuss in great detail with a large number of illustrations the borderline group of breast tumors in the *American Journal of*

Cancer, enough has been recorded here to demonstrate most emphatically that biopsy in breast lesions is rapidly taking the place of clinical diagnosis and gross pathologic diagnosis. In those clinics in which the percentage of women operated upon who consult the clinic for breast troubles, is less than 25 per cent, the number of benign tumors operated upon in that clinic will be more than 50 per cent, and the exploration of the tumor for biopsy may reach almost 75 per cent taking all the tumors together. Therefore, it is not justifiable to explore a breast tumor today unless one is prepared to make and correctly interpret a frozen section. When the tumor of the breast is distinctly malignant the complete operation can then be performed without exploration. Provision should be made in our great medical schools and in the larger hospitals for opportunity for the surgical pathologist responsible in the operating room for the frozen-section diagnosis to study the sections of a large number of these cases in which the result is known, especially for five years or more. And these cases, in addition, should be published with illustrations and the results in our surgical journals.

Method of Biopsy in Breast Lesions.—It was Dr. Halsted's procedure in the very beginning to cut down at once upon the palpable tumor (which, of course, was clinically benign) and to demonstrate at once whether it was encapsulated or not. In the great majority of instances there was exposed an encapsulated adenoma, usually of the adenofibroma or intracanalicular-myxomatous type, much less frequently an encapsulated cystic adenoma. In 1891 Dr. Halsted explored such an encapsulated tumor and sent a piece to Dr. Welch in the pathologic laboratory. However, Halsted was so confident that it was benign that the wound was closed and the operation over before the report was received from the laboratory. This was the first record of a frozen section in the surgical clinic at Johns Hopkins Hospital.

Dr. Halsted, therefore, explored to make a gross diagnosis and not for a frozen section. When there was no capsule he usually found a distinctly and fully developed cancer of the scirrhus or medullary type. It was recognized by the gritty sensation to the knife, by the dots and lines in fibrous stroma, or, in the more cellular cancer, by the material one could scrape with the knife. When this distinct type of cancer was exposed, the surface was swabbed with pure carbolic and a piece of sponge placed in the wound and the skin closed. The complete operation followed. The future follow-up of these cases never brought out any evidence that this procedure was dangerous.

When the area exposed was not encapsulated and not distinctly cancer, it was Halsted's procedure to cut the area out with a good margin of breast tissue and to close the wound. The tumor was then carefully studied microscopically in permanent sections. Both surgeons and pathologists cooperated in the investigation. It was my good fortune to be present at these studies from 1892 on, and we have a complete record of all such instances. Ultimately they were diagnosed cancer microscopically, in spite of the fact that Halsted was unable to see in their fresh appearance any evidence of malignancy. In these cases the glands were never involved. In the majority of instances the complete operation was performed. In a few the patients refused further operation. I published many of these cases in 1920 in the

Archives of Surgery and again in the *Annals of Surgery* for 1930 (The James Ewing volume), and there is now in preparation for the *American Journal of Cancer* an illustrated report on all these borderline tumors.

The remarkable acuteness of Halsted's gross diagnosis is shown in the fact that with but few exceptions, three in all, when he performed the operation in two stages, the ultimate follow-up and restudy have proved that Halsted was right in his gross diagnosis of benignancy.

Today we are having the same difficulty with this group in the frozen-section diagnosis in the operating room.

With rare exceptions, the procedure is different. It seems wiser and safer to deliberately excise the palpable area with a good margin of breast tissue, but not enough to result in a mutilating defect when the wound is closed. This allows the operator to know the character of the surrounding breast tissue and to receive reports on frozen sections before the area is removed. The presence of gross chronic cystic mastitis is evidence against malignancy. The evidence of lactation favors galactocele or lactation mastitis. A definitely senile breast is in favor of malignancy. If one gives the tumor too narrow a margin, cancer nests may be found in the piece excised. When this happens, a piece of gauze saturated with a solution of 50 per cent chloride of zinc should be placed in the wound, the skin incision closed, and the complete operation performed, or one may cauterize the wound with the electric cautery. As there is so much fat in a breast wound, I am inclined to prefer chemical agent. Now, when the mass is excised, it is placed in the hollow of the operator's left hand, best on a towel, and bisected. This gives the best gross view. The encapsulated tumor, the galactocele, the blue-domed cyst, the cyst of the galactocele type are the benign lesions quite easily distinguished in the gross. Fully developed cancer of the scirrhus or medullary type is equally evident to the eye. There is a large group, however, increasing every year, of a nonencapsulated solid tumor the gross appearance of which is deceptive. There are a few in which the gross picture resembles cancer but is not cancer, and a few the naked-eye appearance of which suggests benignancy, which are really malignant. In these cases the frozen section is not so difficult to interpret. The larger number, however, of nonencapsulated areas do not resemble cancer in their fresh appearance, but may offer great difficulties in the frozen and permanent sections.

I have already ventured to record the opinion that in those cases in which the frozen section is not malignant to the pathologist of average experience it is safe to limit the operation to the removal of the tumor, close the wound and obtain the opinion of colleagues in whom the operator and pathologist have confidence.

This new statement is based upon the actual records of our experience in the past two years. This includes breast tissue sent to the laboratory from outside sources, and the patients operated upon in the clinic. It is a reversal of the former rule which advised the complete operation when one is in doubt as to the nature of the lesion.

When one removes a tumor and finds it to be a cyst containing a papilloma, a most careful inspection should be made of the wall of the cyst

beneath the papilloma, and from this area a piece should be taken for frozen section. Sections from the papilloma itself often give a picture suggesting malignancy. However, when the papilloma infiltrates the wall of the cyst into the breast beyond, this is pretty positive evidence of malignancy. One, however, must not interpret multiple smaller minute papillomas in the wall of the larger papillomatous cyst as malignant.

Now and then a smooth-walled cyst shows cancer in its wall, but the contents of such a cyst is always bloody, while that of the benign blue-domed cyst is clear, cloudy, or milk-like.

In spite of all that Dr. Halsted taught me and of my independent experience since, I have found that since 1915 I am becoming more and more dependent on the interpretation of the frozen section in the operating room and rely less and less on the gross appearance of the tissue.

It will be a great advance in the treatment of breast lesions when trained surgical pathologists are more available in the operating rooms of the hospitals of this country. It is not so difficult for them to train themselves to recognize, in the gross tissue or in the frozen section, the malignant lesion of the breast. All others that are doubtful in the frozen section may be treated as benign, the breasts saved until the sections are submitted to one or more experienced surgical pathologists.

Biopsy in Lesions of Bone.—In all the recent publications which I have contributed on diseases and tumors of bone, I have urged that biopsy should be a last resort, except in those clinics in which the pathologist has more than the usual experience in the differential diagnosis of benign and malignant diseases of bone. Drs. Geschickter and Copeland who in the past three years have made such comprehensive studies of the bone material in the laboratory, have come to the same conclusion. It is far safer for the patient to submit the x-ray of the bone lesion to consultation than to perform a biopsy and submit the sections. Nevertheless, in a large number of cases of sarcoma of bone which have lived five years or more after amputation there has been a biopsy without any special precaution as thermal or chemical cauterization and an interval of time between the biopsy, the closure of the wound and the later amputation or resection.

Biopsy for the differential diagnosis of benign or malignant diseases of bone is becoming more and more frequent as a procedure of necessity, because the x-ray diagnosis is becoming more and more difficult when this examination is made earlier and earlier after the first symptom or trauma. I am also of the opinion that it is safer, before biopsy, and while submitting the x-ray films to consultants, to give x-ray treatment at least one full course. We have no evidence that this delay reduces the chances of a cure following the ultimate resection or amputation. Quite a number of our five-year cures have had both biopsy and x-ray irradiation for some months before resection or amputation. We also have a record of a six-year cure of a sarcoma of the tibia of the Ewing type in which there were two biopsies followed by irradiation with radium. In a second case, there was a biopsy of a Ewing sarcoma of the lower end of the fibula followed by treatment with radium. The patient died some four years later from metastasis to the lungs. Clini-

cally and in the x-ray picture, the involved fibula had every appearance of return to almost normal. Therefore it seems wise, when a biopsy is done, and there is doubt as to the pathology, not to resect or to amputate, but to remove as much of the disease as can be done without interfering with the continuity of the bone using the cautery, or, if the lesion is diffuse, removing just enough for microscopic study and cauterizing the surface. The skin wound can then be closed. If the defect is larger, the cavity can be packed with zinc chloride gauze and left open. It is a mistake to explore a lesion of bone without knowing the result of the Wassermann test, or without having tried irradiation first, unless it is distinctly benign or an exostosis, osteomyelitis or tuberculosis. It is also a mistake to explore without having tried irradiation first and having submitted the x-ray films to colleagues for help. However, when one must explore for biopsy, resection or amputation should not be performed unless the microscopic picture is positive.

Difficulties in the Interpretation of Sections Obtained in the Operating Room, of the Earliest Stages of Disease.—Since 1920 I have become more and more convinced not only of the increasing necessity of frozen sections in the operating room, but of the increasing difficulties. In *Surgery, Gynecology and Obstetrics,* (vol. lxix, Nov., 1929, page 717) I contributed an editorial on the Surgical Pathology and Microscopic Cancer in which I ventured the opinion that cancer was becoming more and more frequently a disease which could only be recognized with the microscope, and this accurate recognition was also becoming more and more difficult, and for that reason more cancer students should aid in the search for a differential stain.

I have had in mind offering in the Surgical Pathological Laboratory of the Johns Hopkins Hospital and University microscopic demonstrations as frequently as possible during the year, so that both surgeons, surgical pathologists and those interested in the interpretation of x-ray films of bone, could spend two or three days and be examined on their knowledge, so they would learn, in a most practical way, their abilities to correctly diagnose an x-ray film or a microscopic section of a verified case.

Through the generous financial aid of Mr. Francis P. Garvan, president of the Chemical Foundation, the latter itself and a number of generous patients of my own, it was possible to offer such a course which began in June, 1930, and was repeated in September and December. The fourth meeting will be in April. The response to this invitation was greater than we anticipated and more than our laboratory could accommodate. There seems no doubt that the surgical pathological laboratories of other medical schools must be called upon for aid. Microscopic and x-ray-film demonstrations can be given during medical meetings and can be developed in many other ways to meet the demands.

When to Do a Biopsy.—There are so many hospitals in the country today and so many trained operators that an exploratory incision into almost any part of the body can be performed with safety for obtaining a piece of tissue for microscopic study, and this piece can be sent anywhere for a microscopic opinion. The chief object of this short paper is to emphasize the

dangers of biopsy unless it is preceded by the most careful preoperative study and laboratory investigation, and it is quite just to make the statement that if the operator is in any doubt, he can get help, and the very best, without any cost to his patient, by mail, air mail, telephone or telegram before he proceeds with biopsy. This delicate decision as to biopsy is due to the great enlightenment of the public who are seeking an examination earlier and earlier after the first symptom when the familiar clinical symptoms are absent, when the usual laboratory tests are negative, and when even the x-ray leaves one in doubt. Unless the operator and the pathologist feel capable from their experience to proceed without aid and do not expect to ask for help when they are in doubt as to the microscopic picture, it is far wiser and better for the patient to submit the records of the case with the x-ray if this reveals anything before performing the biopsy. Biopsy for microscopic study is never an operation of urgency.

It is quite possible that there may be developments in biopsy which will make it a much simpler and safer procedure. This I propose to discuss in a second paper.

Two recent cases in the past forty-eight hours illustrate the statement as to the danger of ill-considered and improperly performed biopsy. An adult male wearing an upper plate consults his dentist to repair the bridge on a lower jaw. The dentist observes an abnormal spot on the gum of the upper jaw which he interprets as an irritation from pressure by the plate. The patient is referred to a radiologist for an x-ray. The films show no bone changes. He is then referred to an oral surgeon who refers him to another oral surgeon. The latter removes a piece for microscopic diagnosis, but does not report on this to the patient or his doctor for two months, during which time the wound never heals and a larger ulcer develops. In this case the lesion on the gum could have been completely excised with the cautery or endothermy needle with no mutilation and with a sufficient margin so that in the event of a microscopic diagnosis of cancer nothing further need to be done. Again, the dentist who saw the lesion when he removed the plate should have instructed the patient to discontinue tobacco in all forms and not to wear the plate, and he should have treated him with some mild antiseptic and taught him how to use one. The removal of the cause might have been followed by the healing of the area. During this time the x-rays could have been taken, as they were. If this little wound had not healed within a few weeks, there could have been a consultation as to the first employment of radium; then, if the area had not healed, the complete excision would have followed. We completely excised the area, of course with a much larger margin than would have been necessary at the primary operation, and, finding cancer in the ulcer, had to remove some bone. Should we find cancer in this bone a further operation will have to be performed probably preceded by irradiation. None of this was the patient responsible for. The medical profession missed an opportunity to recognize a possible malignant spot in its earliest stages and remove it with the least discomfort to the patient or loss of time.

In a second case, the patient observed a swelling in the ischio-rectal region. His family physician felt it per rectum, as well as externally. The consultant surgeon diagnosed it as abscess and proceeded to explore it without any preoperative x-ray, Wassermann test, or leucocyte examination. There was nothing acute about the lesion. There was not a single sign of abscess. A few days' delay for investigation would give no discomfort. When the surgeon cut into the mass in the ischio-rectal area it was solid. A piece was removed and found to be sarcoma. Now an x-ray was taken and deep x-ray therapy given. But the original surgeon lost the patient by this precipitated action.

In the *Southern Medical Journal* (vol. xx, Jan., 1927, page 18) there will be found a discussion on biopsy in the diagnosis of malignancy. Here are some of the headings: "In small lesions and small tumors of the skin complete excision without biopsy is the safest procedure." . . . "To repeat again, the danger of incomplete removal is settled. The danger of biopsy when necessary with an interval of time, is not proved. . . . It is my opinion that this group of cases, in addition to breast tumors will force upon the surgical clinics of the world the training of surgical pathologists, to make and interpret, in the operating room, frozen sections."

In *Surgery, Gynecology and Obstetrics* (March, 1927, page 413) a letter from me was published on the Danger of Incomplete Removal of Small and Apparently Innocent Lesions and the Problem of Biopsy. The same subject is again briefly considered in the Transactions of the Section on Pathology and Physiology of the American Medical Association, in 1927. In the *Southern Medical Journal* (vol. xxi, March, 1928, page 179) there is a second presentation of Tissue Diagnosis in the Operating Room which illustrates the table and describes the method which we use in our operating room today.

THE HYDROGEN-ION CONCENTRATION OF THE BLOOD IN UNTREATED CANCER CASES AND ITS RELATION TO PROGNOSIS*

By Gladys E. Woodward, Janetta W. Schoonover, Edith G. Fry, Edward G. Torrance, and Ellice McDonald, Philadelphia, Pa.

THE state of the blood in cancer is of great importance because cancer becomes a systemic disease and cancer cells receive their nourishment from and give off their waste products to the blood. Therefore it is to be expected that the blood of cancer patients should differ from normal blood.

In the study of blood states in cancer and in other diseases also, there are few criteria of value. The blood must be considered as a physicochemical system and, in such a system, the hydrogen-ion concentration (expressed as P_H) is of fundamental importance. Alterations in P_H will control (in part at least) the concentration of various inorganic constituents, the stability of the colloids and, no doubt, the activity of enzymes in the blood and tissues which have an optimum P_H at which they are most effective. The metabolism of the cell in its partition of glycogen to lactic acid and the resynthesis of lactic acid to glycogen has been shown by the work of Warburg and his associates to be different in cancer from normal and such an alteration is influenced (in part at least) in its chemical mechanism by P_H.

Conditions of chemical equilibrium are therefore of greatest importance in deciding the mode of transfer or partition of various substances which are in the blood. As Benjamin Moore, the biochemist, has said, "The most important and fundamental fact in the whole of biology is the delicate and lightly balanced labile equilibrium between the colloids of the cell protoplasm and the osmotic pressure or concentrations of the inorganic ions and other crystalloid constituents," and again, "a normal balance in the ratio of the hydroxyl and hydrogen ions must be maintained or the whole metabolism and life of the cell becomes abnormal and morbid." The estimation of the hydrogen-ion concentration (P_H) of the blood plasma is more, indeed, than a mere measurement of acid-base intensity. Mansfield Clark and his collaborators, in a brilliant series of papers, have shown that oxidation-reduction poise is affected by, and is dependent on P_H or to use a useful chemical term is a function of P_H. Joseph and D. M. Needham have shown that this holds for such cells as marine eggs.

The P_H of the blood is therefore a most important consideration and has attracted the attention of a number of investigators. These studies should be divided into two groups as to time because it was only in recent years that the methods for this determination have been exact and accurate. The

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method of Hastings and Sendroy, a modification of which we have used, was the first practical and accurate method for determination of the P_H of the blood plasma. By means of the hydrogen electrode, Menten, in 1917, was one of the first to claim that "there is to be found an alkalinity of the serum greater than that of the serum of normal individuals." Others have made similar claims at various times.

While the hydrogen electrode is truly reversible in highly refined systems, there is no evidence to indicate that, in such a heterogeneous biologic fluid as blood, the state of reversibility is maintained. Such an active surface as platinum black presents an ideal condition for adsorption with a subsequent catalyzed reaction.

The quinhydrone electrode, an oxidation-reduction system whose single electrode potential depends on the ratio of the activities of quinone and hydroquinone is naturally susceptible to error of great magnitude in poorly defined systems, due to oxidizing and reducing substances and also changes in solubility and reactions catalyzed at the electrode surface. It is impossible to obtain a constant potential with this electrode in the presence of traces of oxygen.

We were not alone in the choice of this method as such good investigators as Errara, Reding and Slosse came to a similar conclusion in a study of the colorimetric and electrometric methods of P_H determination in blood. In addition, Bigwood, Liegeois, Reding and Slosse in a previous investigation state, "Le pH sanguin mesuré à l'électrode á hydrogéne ne parait pas se montrer aussi uniforcement constant que dans le case de mesures colorimetric. Rien ne nous parait justifier definitivement le point de vue que l'electrode á hydrogéne est l'enstrument étálon de la mesure du pH sanguin, au contraire." A misunderstanding of the value and accuracy of the various methods of the estimation of P_H in blood has been the source of much controversy. But it must be stated that after a considerable amount of investigation and study with the advice of a very considerable number of investigators, the two methods of P_H estimation, in blood or in the biologic area, which are, in our opinion, of value are the colorimetric method of Hastings and Sendroy and the glass electrode.

We have in our laboratories made an investigation of the glass electrode; Fosbinder has published the description, and Fosbinder and Schoonover have published results with that method and evidence as to its accuracy. In addition, Fosbinder and Schoonover have made a comparative study of the colorimetric and glass electrode methods and found an average deviation between the two methods of 0.002 or so small as to be negligible and well within the limits of experimental error. Their conclusions are as follows:

1. The colorimetric method of Hastings and Sendroy may be used with accuracy for the determination of P_H of blood and plasma at 38°.

2. Determinations carried out at 38° on 28 samples of diluted plasma from cancerous and normal subjects reveal no discrepancy between the colorimetric and glass electrode methods.

3. The P_H of normal plasma or blood lies between 7.35 and 7.38.

We found we could get consistent and reproducible results with the colorimetric method, but we wished to know whether this method represented the true P_H and whether it could be correlated with the results by the glass electrode. This was proved by the investigations in our laboratories by Fosbinder and Schoonover. The glass electrode would be ideal for P_H estimations of the blood were it not for the difficulties of the method which render it too cumbrous and tedious for routine clinical determinations. The colorimetric method of Hastings and Sendroy is, on the contrary, practical and exact provided the directions for the execution are carefully followed.

Using the colorimetric method of Hastings and Sendroy, McDonald, quite independently, observed that, in *untreated* or advanced carcinoma, the blood plasma was upon the alkaline side of normal and he stated, "In view of this association and alkalinity, it has been interesting to note in my own experience in thirty cases of *untreated or advanced carcinoma* that every one of these had a blood plasma P_H upon the alkaline side of normal (or on the alkaline side of P_H 7.38). It should be noted that these cases were untreated or advanced."

Six months after this, Reding using the same method confirmed McDonald's observations. Reding's first conclusion is as follows: "La maladie cancéreuse se caractérise, outre la tumeur, par de grands troubles humoraux: altération du méchanisme de l'équilibre acide-base de l'équilibre ionique du mechanisme glyco-regulateur" and again he states, "L'alcalose est donc un phénomène constant de la maladie cancéreuse." Reding, as did McDonald, drew attention to the alteration in P_H of the blood produced by treatment, particularly radiation, and to the possibility of the P_H of the blood being used as a biologic check upon the extent and effectiveness of radiation treatment of cancer patients.

It will be noted in this paper that the cases were *untreated* for it is our belief that treatment will often alter the P_H of the blood and indeed that the effectiveness of treatment may be in part at least measured by alterations in the P_H of the blood. Further investigation into this phase is now being done.

Through the courtesy and cooperation of the Radiological Clinic of the Philadelphia General Hospital, patients of the cancer ward and clinic were available for purposes of study. A series of P_H determinations was made upon 65 supposedly untreated cancer cases, but of these only 26 proved, on subsequent examination of their records, to be suffering from a malignant growth, to be absolutely untreated and reasonably free from complications. All of these factors are most important, since the P_H value may be influenced by previous x-ray or radium treatment, by recent medication with buffers (carbonates and phosphates), magnesium salts, etc., or by respiratory affections. Patients, whose diagnoses were questionable and unconfirmed by biopsies or autopsies, were also excluded from our tables. The blood was taken at a uniform time of the day between 10:00 and 10:30 A.M. approximately four hours after breakfast so that the factor of alteration of P_H during the day need not be considered.

RESULTS AND DISCUSSION

Data obtained on the 26 untreated cases considered are listed in Table I, in the order of increasing P_H. Fig. 1 expresses the distribution of the P_H values contained in this table.

TABLE I

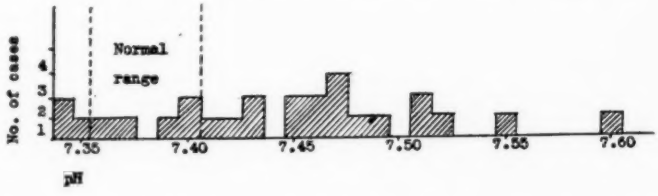
P_H OF UNTREATED CANCER CASES

P_H	NO. OF CASES	CASE NO.	DIAGNOSIS
7.34	2	44	Epithelioma of neck
		48	Carcinoma of antrum
7.35	1	14	Carcinoma of penis
7.36	1	65	Extrarectal carcinoma
7.37	1	60	Epithelioma of floor of mouth
7.39	1	36	Carcinoma of lip and right and left buccal mucosa
7.40	2	38	Carcinoma of buccal mucosa
		13	Epithelioma of ear, bones of face, and glands of neck
7.41	1	21	Sarcoma of humerus (periosteal)
7.42	1	49	Carcinoma of thyroid, metastases to neck
7.43	2	32	Carcinoma of esophagus
		64	Carcinoma of left breast (recurrent)
7.45	2	16	Carcinoma of bladder
		61	Chondroma of right hip
7.46	2	4	Carcinoma of pancreas, metastases to skin of abdomen
		15	Carcinoma of testicle, metastases to inguinal and supraclavicular nodes
7.47	3	5	Carcinoma of breast, metastases to axillary nodes
		6	Carcinoma of cervix, metastases to vagina and parametrium
		20	Sarcoma of right temporal bone and ribs
7.48	1	7	Mixed tumor of parotid gland
7.49	1	17	Mixed tumor of parotid gland
7.51	2	18	Carcinoma of rectosigmoid junction
		30	Generalized carcinomatosis
7.52	1	27	Carcinoma of rectum, metastases to peritoneum and omentum
7.55	1	53	Lymphosarcoma, retroperitoneal
7.60	1	25	Carcinoma of stomach

Average = 7.44; Total = 26.

Fig. 1

DISTRIBUTION OF P_H'S OF UNTREATED CANCER CASES



It will be seen that 77 per cent of the P_H values lie above 7.39, and that the more acid of these are, with the exception of No. 48, superficial cases. The average P_H of the whole group is 7.44, distinctly higher than the normal average which has often been reported to be 7.38 and which we have confirmed by our method. If the figures are divided, the average P_H of the 8 superficial cases is 7.38; that of the 18 internal ones is 7.47, or considerably more alkaline than normal.

It should be remembered by those unfamiliar with the P_H nomenclature that these are not merely decimal shifts but that these figures represent the negative logarithms of the hydrogen-ion concentration so that a shift from P_H 7.38 (or approximately normal) to P_H 7.44 which is our average of blood plasma of untreated cases represents a shift per c.c. of blood of from 0.417×10^{-7} hydrogen ions to 0.363×10^{-7} hydrogen ions or of 13 per cent increased alkalinity in the untreated cancer cases.

The skin or superficial cancers did not give this P_H shift as the average P_H of the blood of 8 *untreated* patients with superficial or skin cancers was P_H 7.38. This was also pointed out by McDonald in 1927 and Reding gives considerable evidence that alkalinity is more pronounced in the internal type or in those cases in which the cancer does not involve the skin or other surfaces.

Alkalosis of the blood plasma of *untreated* cancer patients seems to have a bearing upon the duration of their lives or duration of life is a function of P_H. This paper was delayed a number of months for the correlation of the colorimetric method and other studies of P_H and this delay allowed us to make the observations in Table II which shows the relation of alkalinity of the plasma and prognosis. In the series, the more alkaline the blood plasma of untreated cases the worse the prognosis. Warning should be given in applying this rule to patients whose P_H may be modified by treatment, complications or medication.

Table II shows the apparent relation of P_H to prognosis.

TABLE II

RELATION OF P_H TO PROGNOSIS

CASE NO.	DURATION OF LIFE AFTER UNTREATED P_H	UNTREATED P_H
48	15 months*	7.34
44	12 months*	7.34
15	12 months*	7.46
60	8 months*	7.37
38	(Unknown)	7.40
14	15½ months	7.35
16	10½ months	7.45
61	10½ months	7.45
65	9½ months	7.36
36	9½ months	7.39
64	6½ months	7.43
21	5 months	7.41
32	3½ months	7.43
13	10 weeks	7.40
20	8 weeks	7.47
4	7 weeks	7.46
7	7 weeks	7.48
49	6 weeks	7.42
5	6 weeks	7.47
18	5 weeks	7.51
17	3 weeks	7.49
27	12 days	7.52
53	12 days	7.55
30	10 days	7.51
6	9 days	7.47
25	3 days	7.60

*Still living. No. 15 reduced to P_H 7.33 after treatment.

Table II contains the values listed in Table I revised with respect to the time that the patient lived after the first observed P_H. The first five subjects, so far as is known, are still living, but the time noted in the second column does not represent the interval between the date of the "untreated" P_H and the present. For these five, this figure denotes the time between the patient's first P_H value and the time he was last seen by the hospital authorities. The remaining twenty patients have all died.

Three of the five living patients had an initial P_H upon the acid side of normal while that of No. 15 was reduced to 7.33 after treatment. With the exception of Nos. 14 and 65, it will be seen that all of the patients who died had an alkaline P_H before they were treated. It is apparent that increase in alkalinity corresponds to a shorter duration of life. This statement is supported by the fact that Nos. 14 and 65, both of whom had an acid P_H, survived for fifteen and one-half and nine and one-half months, respectively.

This table indicates that an alkaline P_H in cancer patients' blood, not only means an unfavorable prognosis, but that, in this series, a greater degree of alkalinity means a shorter term of life. These facts may be accounted for by the type, location or degree of progress of the cancer, but nevertheless, prognosis from the P_H findings is, in the majority of cases, successful.

We wish to express our gratitude to the authorities of the City of Philadelphia and of the Philadelphia General Hospital for making this study possible. We also wish to thank Miss Rachel G. Franklin, chemist, for her assistance.

We include a list of 25 P_H determinations upon adults carefully scrutinized as to their normality and examined under standard conditions with

TABLE III

LIST OF NORMAL P_H VALUES

NO.	NAME	DATE	P_H
1	C.W.E.	Nov. 24, 1928	7.36
2	J.W.Sc.	Feb. 14, 1929	7.37
3	G.E.W.	Jun. 10, 1929	7.38
4	E.G.T.	Jun. 11, 1929	7.39
5	J.W.Sm.	Mar. 6, 1930	7.38
6	E.B.S.	Mar. 14, 1930	7.38
7	M.Sc.	Mar. 14, 1930	7.39
8	G.S.H.	Mar. 15, 1930	7.38
9	M.I.D.	Mar. 18, 1930	7.38
10	E.E.S.	Apr. 4, 1930	7.39
11	W.W.McC.	Apr. 23, 1930	7.37
12	M.E.A.	Apr. 25, 1930	7.38
13	G.N.	May 7, 1930	7.39
14	M.T.	May 16, 1930	7.38
15	C.d.R.	June 26, 1930	7.39
16	M.Q.	July 29, 1930	7.39
17	M.C.	Aug. 14, 1930	7.39
18	E.S.	Aug. 19, 1930	7.38
19	W.N.	Oct. 20, 1930	7.38
20	J.R.M.	Nov. 19, 1930	7.38
21	A.S.	Nov. 21, 1930	7.39
22	W.C.H.	Jan. 19, 1931	7.40
23	A.S.G.	Jan. 20, 1931	7.36
24	A.R.S.	Jan. 20, 1931	7.37
25	E.G.F.	Jan. 20, 1931	7.40
		Average =	7.38

an average P_H of 7.38, which we consider the normal datum line. In order that there may be no misunderstanding as to the method of Hastings and Sendroy and our modifications of it, the complete directions are appended to this article.

SUMMARY

1. The average P_H of 26 untreated cancer patients was found to be 7.44 or considerably more alkaline than normal.

2. The average of 8 untreated superficial cases was 7.38; that of 18 untreated internal cases, 7.47.

3. The degree of alkalinity is closely related to the prognosis of the disease.

METHOD

A. *Preparation of Standards.*—Twenty-four Pyrex test tubes, 18 × 150 mm., of exactly the same bore as that of the 18 × 100 mm. tubes used for the blood sample (see next section) are selected for the standards. These are selected as follows: 10 c.c. of water is added to several of the tubes and the levels compared to the average 10 c.c. levels of the 100 mm. tubes. The one which exactly matches is taken as a standard for selecting the other 23. In choosing these it is better to compare the 25 c.c. levels since any difference is magnified 2½ times that at the 10 c.c. level. Therefore 15 c.c. more is added to the first tube selected, the other tubes filled to the 25 c.c. levels and those chosen in which the height of the column does not vary more than ¼ inch from the standard for selection. The necessity for this, as well as the need for then arranging the tubes in pairs in the order of ascending levels, placing the best tube in the center of the range, has been emphasized in a recent publication from this laboratory by Schoonover and Woodward.

The table used in filling the tubes is taken from the work of Hastings and Sendroy and is so calculated that the P_H tubes which are to be matched by them must be at 38° C. at the time the comparison is made.

TABLE IV

MEASUREMENTS FOR PREPARATION OF STANDARDS

P_H	ALKALI TUBE		ACID TUBE	
	0.0075% PHENOL RED*	0.01 N NaOH	0.0075% PHENOL RED	0.001 N HCl
	c.c.	c.c.	c.c.	c.c.
7.15	0.60	24.40	1.90	23.10
7.20	0.65	24.35	1.85	23.15
7.25	0.71	24.29	1.79	23.21
7.30	0.77	24.23	1.75	23.27
7.35	0.84	24.16	1.66	23.24
7.40	0.90	24.10	1.60	23.40
7.45	0.97	24.03	1.53	23.47
7.50	1.04	23.96	1.46	23.54
7.55	1.11	23.89	1.39	23.61
7.60	1.18	23.82	1.32	23.68
7.65	1.25	23.75	1.25	23.75
7.70	1.32	23.68	1.18	23.82

*For the preparation of the solutions necessary see Section E below.

The 0.0075 per cent phenol red is measured from a 10 c.c. certified burette, graduated in 0.05 c.c., into the labeled standard tubes, pair by pair, because the amount added to the second tube of the pair always completes a total of 2.5 c.c. This facilitates measurement of the dye, and accuracy in this procedure is exceedingly important. The alkali or acid is then run in, one drop of toluene added to act as a stabilizer, the tube closed with a rubber stopper and inverted to mix it.

New 0.0075 per cent phenol red and new sets are made up once a month and compared to the previous set in order to check up on any error in measurement in the new set. It has been shown previously that sets keep perfectly for at least a month (Schoonover and Woodward). In fact several sets which have not been exposed to the light to any great extent have shown *no change* during three months.

B. *Preparation of Indicator and Control Tubes.*—Pyrex test tubes, 18 × 100 mm., made specially at the Corning Glass Works (order No. L-2388) for uniform bore, are used for the indicator and control tubes. These are cleaned by boiling in lux and then 1 per cent hydrochloric acid. The tubes must be thoroughly rinsed with distilled water and dried before use.

The indicator is adjusted to P_H 7.40 with alkaline indicator added drop by drop (for these solutions, see Section E). This cannot be measured out from a pipette since the CO_2 from the breath changes the P_H of the indicator. A 5 c.c. syringe is used, 4 c.c. being measured into a dry 100 mm. tube. Neutral paraffin oil is immediately added to make a layer 1.5 to 2.0 cm. If the color after a few minutes is found to be below 7.35 or above 7.45 the tube is discarded.

A control tube is prepared by adding 4 c.c. of normal (0.9 per cent) saline to another of the 100 mm. tubes.

C. *Collection of the Blood Sample.*—Blood samples are drawn into a 1 c.c. Vim tuberculin syringe. This, sterile and dry, is fitted with a 21-gauge needle and sterile normal saline solution drawn in. Air is expelled and the syringe emptied, leaving the needle and tip of the syringe filled with saline. This procedure eliminates the air bubbles invariably present when an empty syringe is used.

The area over the vein is cleansed with alcohol and a tourniquet applied. As soon as the needle enters the vein the tourniquet is released and the blood is drawn into the syringe (0.1 or 0.2 c.c.) and expelled back into the vein several times. This washes out the saline, and at the same time allows the stasis induced by the tourniquet to disappear. The syringe is then filled to the 1 c.c. mark, withdrawn, and the needle immediately inserted under the oil in the indicator tube. Two-tenths of a c.c. (0.2 c.c.) is expelled into the indicator and the needle then transferred to the control tube into which another 0.2 c.c. is expelled.

D. *Reading of the P_H.*—After the 0.2 c.c. of blood is added, each tube is rotated gently until the blood is mixed throughout. Both tubes are then centrifuged at a low speed (about 1500 r.p.m.) for 4 min. to force the corpuscles to the bottom of the tubes. The indicator tube is then placed in a beaker of water at about 42° C. At the end of about 3 min., if, and only if, the temperature is 39 or 40°, the tube is removed and placed in the comparator block in the place indicated in the diagram below. It is assumed that by the time a reading is made the tube will have cooled to blood temperature, 38°. The control and standard tubes are likewise placed as in the diagram, the standards being changed until the nearest color match is obtained.

	Alkali tube	Acid tube	Blood-saline control tube	
Eye				Light
	Blood-saline indicator tube (38°)	Water tube	Water tube	

The following is an example of the method of final decision on a reading. Suppose a sample of blood seems to be about 7.40. The 7.35 tubes are tried and seem quite yellow, or low. The 7.40 tubes are a trifle too high. Now we avail ourselves of a new combination, the 7.35 pink and the 7.40 yellow tubes. These do not give a P_H of 7.375, but rather one of 7.37, because they do not quite have a total of 2.5 c.c. of 0.0075 per cent phenol red. This fall in concentration is so slight, however, that it serves, even to the trained observer, as a diminishing of the pink color, and hence is an excellent match for a 7.37 sample. In this case 7.37 is lower than the 7.40 is high, so that our sample has a P_H of 7.39.

The combination of the low pink tube and the next higher yellow tube may be used all along the range, always calling the resulting P_H 0.02 higher than that marked on the pink tube.

In case a reading takes over a minute or so, the indicator tube is replaced in the water-bath and brought back to 38° before making the final decision on its P_H.

E. *Solutions.*—0.0075 Per Cent Phenol Red: As a stock solution we have used a product of Hynson, Wescott and Dunning, 1 c.c. of which contains 6 mg. of dye. By diluting 6.25 c.c. of this to 500 c.c. with freshly distilled water, the 0.0075 per cent solution is obtained.

0.01 *N* Sodium Hydroxide: This need be only approximate. If a stock of normal or tenth normal solution is at hand, it may be made by dilution of these 1 to 100 or 1 to 10 respectively. If no stock is at hand, the tenth normal which is then diluted as above is made by dissolving 4.0 grams of NaOH in one liter of distilled water.

0.01 *N* Hydrochloric Acid: This also need be only approximate. It should be made by diluting tenth normal acid 1 to 100. If no tenth normal acid is at hand this may be made by diluting 8 c.c. of conc. HCl (sp. gr. 1.18-1.19) to one liter.

Phenol Red Saline Indicator: 52.5 c.c. of 0.0075 per cent phenol red and 4.5 grams of sodium chloride are placed in a 500 c.c. volumetric flask and diluted to about an inch below the mark. The color is yellowish. It is brought to a slight pink by adding 0.1 *N* NaOH drop by drop. When the correct color, P_H 7.40, is obtained, it is further diluted with water to the mark.

Alkaline Saline Indicator: 10.5 c.c. of 0.0075 per cent phenol red and 0.9 grams of sodium chloride are placed in a 100 c.c. volumetric flask and diluted to the mark with the 0.01 *N* NaOH.

Normal Saline: 9 grams of sodium chloride are dissolved in one liter of distilled water.

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CANCER AND THE LABORATORY*

By Ellice McDonald, M.D., and William C. Hueper, M.D.,
Philadelphia, Pa.

I. THE CLINICAL LABORATORY (BY ELLICE MC DONALD)

THE consideration of cancer is not a single problem. The changing course of the modern treatment of cancer requires more than the casual attention of the surgeon. New methods, such as radiation, require new laboratory examinations and these are most important before any treatment can be instituted to control the course of that treatment. One important sentence should be placed over the door of every cancer clinic: *the first treatment of the cancer patient determines the result.* This holds whether the treatment is surgical or radiologic. If the surgical treatment is not properly determined and executed, the result is well known. If the proper radium or x-ray treatment is not carefully executed, the tumor is often so affected as to be resistant upon a succeeding treatment. No physician may dally with injection or other treatment of a cancer patient for three months and then turn that patient over to the cancer clinic to be treated and expect to have good results.

For these reasons, it is most important that a cancer patient should be carefully studied before treatment and without delay, and that the concomitant conditions in the patient should also be examined during treatment. This is impossible without the close cooperation of the laboratory. In addition, in considering the treatment of cancer patients, as we do in a group clinic or conference, there should be in attendance the pathologist and other laboratory people to properly evaluate the results of various tests that have been done, and to suggest other laboratory examinations before any definite line of treatment is chosen.

The ideal is a blood or specific laboratory test for the diagnosis of cancer. This counsel of perfection seems not impossible, but as yet it has not been attained except, of course, by microscopic examination of sections of the growth. In our laboratories, we have made a survey of 35 different blood, serum, or other laboratory diagnostic tests for cancer. Of these, we have made a more particular study of five methods, which have been advocated for a diagnosis of malignant disease from a study of the composition and behavior of the blood. Unless such a test is 100 per cent accurate, it is valueless, and none have been satisfactory in our hands. We paid particular attention to the Abderhalden reaction with its more recent modification, the interferometric method, the Shaw-McKenzie test, and the Fry test. Our results with these tests have not been sufficient to encourage us to continue the investigation. Others have come to similar conclusions; in fact Haynes and Wolf suggest that much of the observed

*From the Cancer Research of the University of Pennsylvania Graduate School of Medicine.

effect in the Abderhalden interferometer test for malignancy is due to the soluble substances coming from the rubber stoppers which close the incubation tubes, for, when these are coated with sterile tin foil most of the differences which have been observed, disappear. Suffice it to say, that in our experience we have found no blood test for cancer sufficiently satisfactory to continue investigation upon it. This does not mean that we have investigated them all, or that a blood test in cancer is not a possibility.

While the laboratory can help but little in the exact diagnosis of cancer, except through biopsy, there are certain tests which are very necessary before any treatment is instituted and useful in making a differential diagnosis. The usual Wassermann test should be a routine, and carefully checked by different methods. No mouth lesion should be dismissed from the cancer category because of a positive Wassermann, as the two diseases are so frequently concomitant.

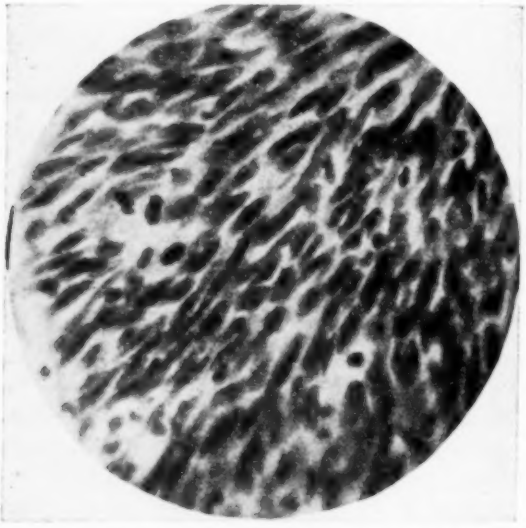

Fig. 1.—Spindle cell carcinoma of the uterine cervix (high magnification).

The blood count should be done on every patient before any treatment, particularly before any radium or x-ray treatment is undertaken. A leucocytosis, even of a moderate degree, is an indication for special attention in the preliminary preparation before radiation. A leucopenia or a lymphopenia with the normal number of leucocytes is an indication for cautious treatment. Measures should be taken for cautious treatment before irradiation and a somewhat smaller first dose should be given. The blood condition should be carefully investigated before repeating the dose. If an unfavorable change is found in the blood after the first irradiation, the second application should be delayed and a slightly smaller dose given. A slight lymphopenia is not infrequent in cancer cases and is seldom increased by radium treatment. A diminution in the polymorphonuclears and the lymphocytes is the first significant effect of radium when the original count is approximately normal. A leucocyte count should be done before every radium treatment.

The biochemical tests which are necessary will vary with particular opinion. In our laboratories, we pay particular attention to the hydrogen-ion concentration (P_H) test of the blood, which we have studied now for four years. In our opinion, in *untreated* cancer cases, the P_H of the blood plasma, as done by the method described by another article in this number, is some measure of the prognosis of the disease. It should be noted that these are *untreated* cases, as it is our belief that treatment may alter the P_H. The test is easy to do but the directions should be carefully followed. In *untreated* cancer cases, in our experience, the P_H of the blood plasma is relatively more alkaline than normal, the greater the alkalinity, the worse, in our experience, is the prognosis.

Our experience is not yet sufficient to say that a similar thing holds in treated cases, but we have come to regard the very alkaline P_H (7.45-7.60) as a bad sign, and this particularly so when the P_H goes more alkaline after ir-

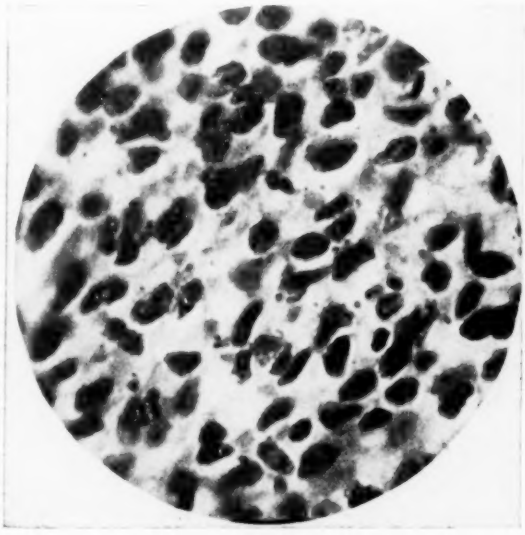

Fig. 2.—Round cell carcinoma of the uterine cervix (high magnification).

radiation than it was before treatment. Investigations into this latter phase are, however, still under way. The P_H test (as carried out by the method described in this number) has considerable promise of being a valuable check upon radiation treatment. Exactly as with a leucocytosis, measures should be taken to improve the condition and, if possible, restore the P_H toward normal before giving irradiation. A check should be made after the first irradiation to show any results, and another P_H should be done before any succeeding irradiation. If the reaction is markedly toward alkalinity after the first treatment and does not recover before the time for the second treatment, again measures should be taken to improve the general conditions toward normal, and the amount of the second irradiation carefully considered. Certainly in the face of such circumstances, large doses of radium or x-rays should not be given.

The next most important blood test in cancer is the blood sugar. There seems to be evidence that a lessened glucose tolerance is characteristic of cancer, and the less the tolerance the worse the prognosis. The glucose tolerance re-

quires, however, four blood-sugar determinations and four withdrawals of the blood in one morning, and is too formidable, a procedure for a routine. Considerable information may be obtained before any treatment by a single blood-sugar examination and this should be a routine. Again, if the blood sugar is higher before the second irradiation treatment, care should be taken.

The P_H and blood-sugar tests are, in our opinion, of great importance in cancer study and treatment, and in our laboratories, are routine. They have possibilities of being of great clinical importance in cancer treatment.

We have experimented with numbers of other biochemical tests. Total calcium determinations, while not absolutely routine with us, are very frequent. Urea estimation is, of course, indicated in many kidney and bladder conditions, and again a rise in the urea estimation after irradiation is not a good sign, although we have not had such experience with it as we have had with the P_H and blood-sugar tests.

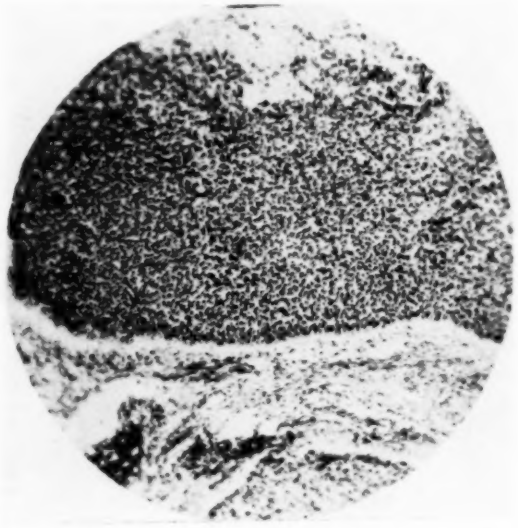

Fig. 3.—Round cell carcinoma of the uterine cervix (low magnification.)

Other biochemical tests are under investigation in our laboratories and there are, at least, three more which have considerable promise of being of value in cancer study and treatment. Any test, to be of value, should give consistent and reproducible results and be capable of being correlated with the continuing clinical course of the patient's disease, and be of value in throwing light upon the conditions of the vital system and its reflection to treatment, or alteration in treatment. One difficulty, in the chemistry of the blood, is that methods which give good results in the chemical laboratory, when carried over to the biologic area of the heterogenous system of the blood, with its proteins and colloids, often fail in accuracy. Then again, in blood, large quantities are not available and the amount of substance to be estimated is usually very small. For these reasons, any method to be put into clinical practice must be carefully scrutinized and checked.

Biochemical study of the blood in cancer, however, has great possibilities of throwing light on the course of the disease. After all, cancer lives in the body and the blood may be considered the environmental fluid of the cells. Alterations in the environment will produce alterations in the functional activities of the cell. Cancer has so long been considered a cellular disease that we have been apt to forget that the cell and its environment are one and that no investigation of cell is complete without a study of its environment. When we say we have had fish for dinner, we misstate the fact; we have had something which, when it was in its environment, the water, was a fish, but, removed from there, is a dead thing. When we study the cancer cell through the microscope, we study a dead and altered thing, removed from its environment, having lost its functions and with most of its qualities destroyed.

The study of these functions and qualities by means of the investigation of the environmental conditions, of which the blood is a great part, offers con-

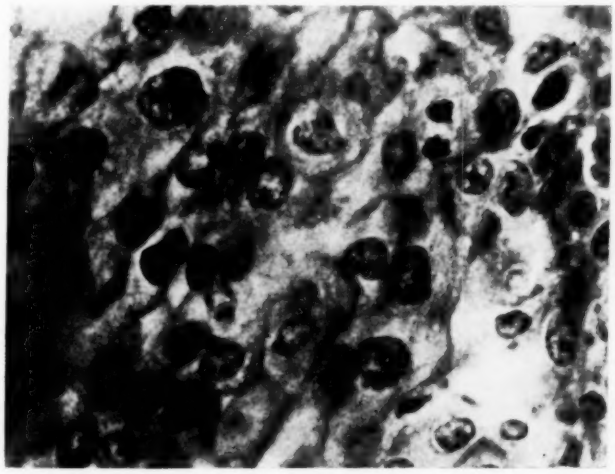

Fig. 4.—Spinous cell carcinoma of the uterine cervix (high magnification).

siderable promise. In such studies, it should not be forgotten, while the ideal is the discovery of the cure of cancer, that improvements of existing methods of treatment are a second duty, not less important than the ideal. Present methods of treatment in cancer are not without result when the total percentage of cures are compared with such diseases as pneumonia, etc. It is true that none of us are satisfied, because we realize that present methods are merely a bridge leading to better things. Still, if these present methods can be improved, and their value extended, it will be a great and immediate help. Of studies which may lead to that consummation, devoutly to be wished for, investigation into the chemistry of the blood in cancer patients seems to be a field which has great promise of helpful results.

PART II. PATHOLOGY OF MALIGNANT TUMORS (BY W. C. HUEPER)

In the relations between clinician and pathologist in regard to neoplastic diseases, the histologic diagnosis plays the preeminent rôle. It is therefore

natural that both parties should be equally interested to assure and improve upon the reliability of the histologic diagnostic methods of tumors. The reliability of these procedures depends on numerous factors which are partly controlled by the surgeon, partly by the pathologist.

The factors depending on the surgeon are, briefly outlined, as follows:

1. Biopsy tissue must be removed from suspicious parts of a lesion. Negative histologic reports in the presence of strong clinical suspicion of malignancy are an indication for a repetition of the diagnostic excision. The greatest difficulties in the selection of the proper place for a biopsy are usually encountered in infiltratively growing, not ulcerating cancers.

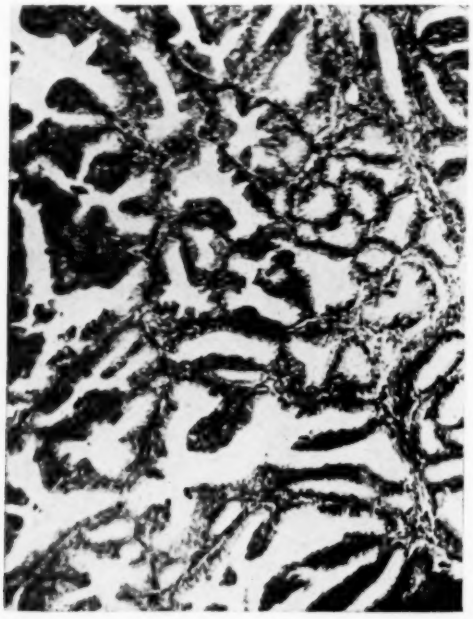

Fig. 5.—Malignant adenoma of the uterine cervix.

2. The tissue removed must include the peripheral portions of the tumor, e.g. the edge of an ulcer and not only its base, because the periphery contains the actively growing sections of the neoplasm with the least amount of secondary aging processes and is therefore best suited for the determination of the actual character of the growth.

3. The excision must not be made parallel to the surface resulting in the removal of a more or less thin slice of superficial tumor tissue, but must be a wedge including the deeper tissue where infiltrative growth may occur.

4. The amount of tissue removed must be sufficient for the application of the technical methods and for the adequate histologic study. Atypical cellular groups suggesting malignancy may also occur in inflammatory proliferations and so-called precancerous lesions. Biopsy methods with very small amounts of tissue such as removed by needle or trocar have therefore definite limitations as to their reliability.

5. The tissue must be placed directly after removal into a fixing fluid to prevent decomposition and drying, processes which may later interfere with the technical preparation of the sections and the interpretation of the histologic findings.

6. The specimen must be accompanied by sufficient clinical data to enable an intelligent interpretation by the pathologist.

The importance of this requirement, which is often neglected, cannot be too strongly emphasized. The pathologist can expect that the following data accompany the specimen: Name of patient, sex, age, location of tumor, relation to adjacent tissues, treatment given previous to excision, date of last menstruation and pregnancy in gynecologic cases, blood picture in cases of lymph node

Fig. 6.—Papillary adenocarcinoma of the uterine cervix.

specimens, rapidity of growth. Several examples may help to illustrate this point. The age is, for instance, very important in the estimation of the malignant character of a certain type of adenomas of the thyroid. While these tumors are usually benign, if occurring in persons 20 to 30 years of age in spite of some histologic features indicating malignancy, they have definite malignant qualities, if they are present in persons of cancer age, that is, above forty years. The data in regard to the location of the tumor may influence in a similar fashion the interpretation of the histologic findings. Epithelial proliferations in the submucous tissue of the urethral caruncle which resemble very closely similar but definitely malignant proliferations of other organs are clinically benign and do not represent small cancerous growths. It is, moreover, necessary to give definite data in regard to location, if several excisions are made,

so that the pathologist is able to report on each one separately. This is for instance important if a curettement of the uterine fundus and cervix is made.

The finding of decidual structure in curettements of women with postclimacteric hemorrhages points to the existence of an ovarian cancer and emphasizes the importance of the information in regard to the data of last menstruation. Also, in the differential diagnosis of chorioepithelial proliferations, the date of the last menstruation is absolutely necessary to decide upon the malignant or nonmalignant character of the cells as the chorionic proliferations of the first three months of pregnancy are more or less identical with those found in choriocarcinoma. Rapidly growing breast tumors in young pregnant women arouse sometimes the suspicion of malignancy and even the histologic picture of the removed tumor may be confused with adenocarcinoma as there is evidence of a marked proliferation of indistinctly outlined glandular formations lined by rather large hypertrophic cells. But, on closer scrutiny, it is

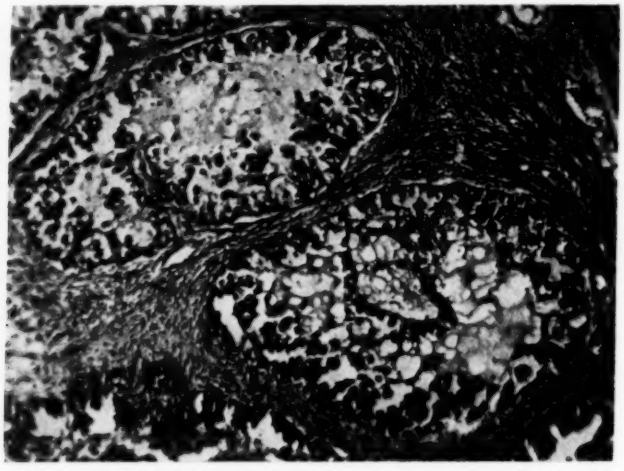

Fig. 7.—Gelatinous adenocarcinoma of the uterine cervix.

usually found that the tumor represents a benign fibroadenoma which shows the signs of pregnancy hyperplasia. Atypical epithelial proliferations and decidua cell nests are found during, and for some time after, pregnancy in the cervical portio and may become the cause of incorrect histologic interpretations in the absence of proper clinical data.

The knowledge of the blood picture is often absolutely essential in the differential diagnosis of small round cell sarcoma and leucemia of lymph nodes.

Roentgen and radium rays, as well as the use of the radium knife, cause pathologic changes and distortions in the cells and may be the cause of incorrect histologic diagnoses especially by pathologists not well familiar with the effects of these agents upon cellular structure.

One important factor under the control of the pathologist which is apt to impair the reliability of the method of histologic diagnosis is that the interpretation of the pathologic-histologic terms used by the pathologist differs from that given them by the clinician. In an efficient histologic-diagnostic service, the

histologic description and diagnosis should not leave any room for misunderstandings which may have sometimes rather unfortunate effects upon the type of treatment.

A scientifically well founded and clinically practical classification and nomenclature of tumors must, therefore, be regarded as one of the fundamentals upon which the reliability of the tumor diagnosis and comparative studies of tumor pathology and therapy are built. Blastomas may be classified upon the following principles: (1) Location of the tumor; (2) physical properties; (3) etiology; (4) histology, (a) histiogenetic principle, (b) embryological principle, (c)morphologic principle, (d) principle of parenchyma-stroma ratio, (e) biologic principle.

Each of these principles on which a classification may be based will convey only a certain restricted amount of information and characterization of the

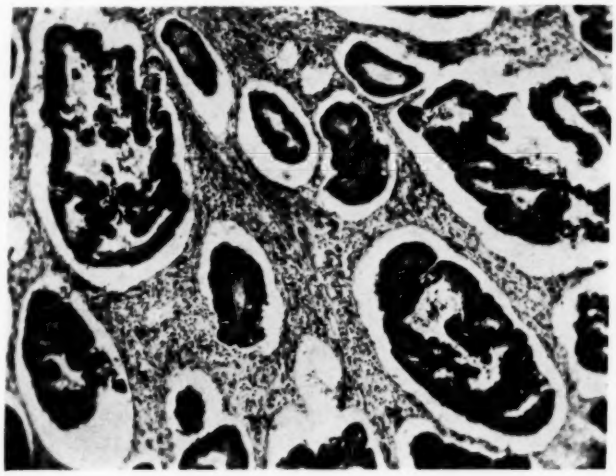

Fig. 8.—Simple adenocarcinoma of the uterine cervix.

tumors thus classified. Each one of them has its definite advantages, if used in the limited field and purpose for which it is designed, but will reveal its defects when applied to general clinical and pathologic use. While a classification based upon the histologic principle will serve best as the fundamental method of grouping neoplasms for practical clinicopathologic use, terms reflecting the principles embodied in the other above mentioned classifications have frequently to be added to the designations of the histological classification to obtain an adequate characterization of a neoplasm. The terms used must be descriptive, defined and brief. Complex names can sometimes not be avoided for the proper characterization of certain tumors. The use of authors' names must be regarded as objectionable in a scientific classification, as such names do not fulfill the basic requirements of a medical term and may become, moreover, the source of great confusion, as the name of the same author may appear in the designation of several widely differing diseases (Paget, v. Recklinghausen, etc.) and also as the names of different authors are used for the same disease in different countries.

1. The regional classification of tumors according to their seat is a necessary one, but gives very little more information beyond this scope.

2. The physical classification which is based upon the physical properties of blastomas may group them according to various features, such as solitary and multiple, solid and cystic, intrinsic and extrinsic, encapsulated and nonencapsulated, circumscribed and diffuse, ulcerative and indurative, papillomatous and polypous, colored and noncolored, soft and hard, central and peripheral, nodulated and nonnodulated, etc. While all these characteristics may help in the more accurate definite and description of a tumor they offer very little of actual value without the background of a histologic classification.

3. The etiologic classification does not render a sound basis for grouping tumors at the present highly defective state of our knowledge of tumor etiology.

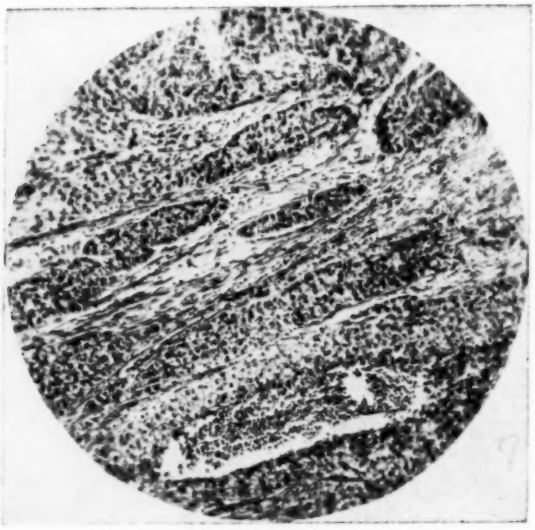

Fig. 9.—Solid adenocarcinoma of the uterine cervix.

4. Histologic classification:

a. The histogenetic classification is based upon the assumption that each of the various types of tumors is derived from a definite and in its character fixed type of normal cell. While a classification on this basis appears to be a rather simple and scientifically sound one, its practical application in the individual case encounters not infrequently great difficulties on account of the factors of tissue misplacements, metaplasias and morphologic resemblance of different types of tumor tissues (myxoma-chondroma-chordoma-gelatinous adenocarcinoma or carcinoma-sarcoma-endothelioma). On the other hand, a tumor may pass through various structural phases which may obscure the tracing of its histogenesis. An adamantinoma may for instance lose its characteristic structure and change into a nonspecific cylindrical cell carcinoma. The availability of small amounts of tissue (biopsy material) will often interfere with a proper tracing of the histogenetic origin of the tumor.

b. A classification on the embryologic principle, that is, upon the origin of the tumor tissue from elements of the three germinative layers (ectoderm,

endoderm and mesoderm) is highly impractical as our embryologic knowledge is still rather defective and moreover unsettled in many respects and as the adoption of the embryologic principle would necessitate the creation of an entirely new type of nomenclature.

c. The morphologic classification is developed on the principle of tissue resemblance. This factor can be more or less easily determined in tumors by comparing their structure with that of the existing embryonic and adult tissues. Due allowance must be made in the comparison of malignant tumors with normal tissues to their tendency to produce pathologic deviations of morphology. While it will not infrequently be possible to recognize in malignant blastomas a definite morphologic resemblance to existing normal organic structures, the neoplasms will sometimes reveal only a pattern of more general character such as a more or less indistinct glandular structure on the basis of which a grouping

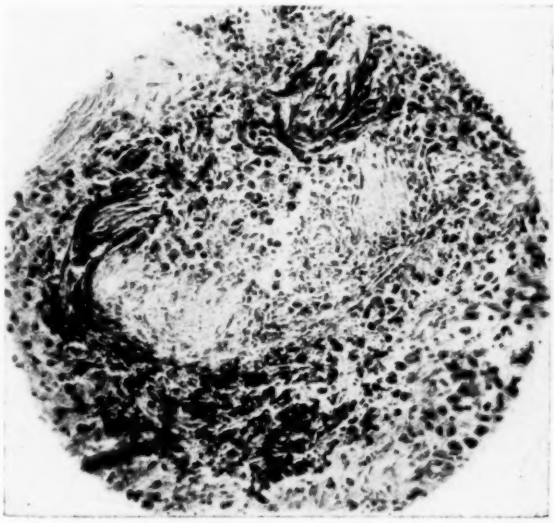

Fig. 10.—"Pencil-cells" surrounding a central necrosis in a testiculocarcinoma (seminoma).

may be attained. The great majority of blastomas can be classified on this basis without great difficulties. But one has not entirely eliminated the embryologic principle in the nomenclature of this classification, if one insists upon using existing terms. This is especially true in regard to the terminology of the malignant tumors. While rather neutral terms such as glioma, chordoma, hypernephroma, etc., are used for the benign types, words, like carcinoma, stand in general for the malignant epithelial neoplasms and sarcoma for the malignant blastomas of mesodermal derivation. But this rule is not always strictly adhered to as for instance the malignant tumors of the nervous tissue which is of ectodermal origin are called sarcomas, while some of the benign and the malignant tumors of the kidney which is of mesoblastic origin are called adenomas and carcinomas. The infraction from this rule will become even greater, if one should accept the claim to the mesoblastic origin of the ovary, tube, uterus, testicle, etc.

d. Classification on the basis of stroma-parenchyma ratio. The tumors are classified according to the amount of parenchyma and stroma respectively present. Terms, such as medullary, encephaloid, molle, etc., added to the name of a tumor designate that it consists mainly of tumor parenchyma, while terms such as scirrhous, fibrous, etc., are generally used to describe the fact that the blastoma is predominantly composed of stroma. The word "simplex" is by some authors used to characterize an intermediate group of blastomas in which neither of the two composing elements seems to be in the prevalence. While the term, fibrous, is an appropriate and correct designation for the abundance of stroma, the word scirrhous (hard), medullary (marrow like in consistency),

Fig. 11.—Gelatinous adenocarcinoma of intestine. Signet ring type.

encephaloid (brain like in consistency), molle (soft), etc., imply the thought that a reliable conclusion can be drawn from the consistency of a neoplasm upon its paranchyma-stroma ratio. Such an assumption is, however, entirely incorrect in many instances, as necroses, edema, cyst and secondary scar formation may affect considerably the actual consistency of a tumor to such an extent that the cellularity of the characteristic parts of a neoplasm may be completely misjudged from a palpatory examination.

Terms which necessitate such unreliable assumptions are therefore better avoided and replaced by more correct and appropriate descriptive terms as for instance edematous fibroma, cellular fibroma, fibrous carcinoma, etc. It may be advisable to point out here one precaution which has to be taken, if the

parenchyma-stroma principle shall be correctly applied. It must be remembered that a correct evaluation of the structure of a tumor can be accomplished only if the characteristic and vitally active peripheral parts of a neoplasm are used to this end. Structural deviations between the peripheral vital parts of a tumor and the central degenerating, often fibrosing, portions of a tumor are common and account for the frequently noted statement that blastomas are not infrequently cellular (medullary) in one portion and fibrous (scirrhous) in another portion.

For my own use the following classification has been developed, based upon the morphologic principle, which was made the guide and consideration to other factors subordinated.

Fig. 12.—Pencil-cells in papillomatous adenocarcinoma of the rectum.

While most of the terms are self-explanatory, it is advisable to comment upon the meaning of some of the terms. The name "adenocarcinoma" is used in a broad sense, including all tumors showing a glandular pattern regardless of the fact, whether the lining cells are of columnar, cuboidal or more or less irregular shape.

The terms "carcinoma" and "sarcoma" designate blastomas of widely varying degrees of malignancy. They include tumors which show usually only local infiltrative and destructive qualities (basal cell carcinomas of the skin, some fibrosarcomas, argentaffinocarcinoma of the intestine) on the one side and the neoplasms of highly malignant properties with rapid growth and early

TABLE I

CLASSIFICATION OF BLASTOMAS

TISSUE TYPE	BENIGN TYPE	MALIGNANT TYPE
A. *Indifferent Embryonic Tissue*		Malignant indifferent cell blastoma (Malignoma)
B. *Connecting and Supplying Tissues*		
1. Embryonic mesodermal tissue		Polymorphous cell sarcoma
		Round cell sarcoma
		Spindle cell sarcoma
2. Fibrous connective tissue	Fibroma	Fibrosarcoma
3. Cartilagenous tissue	Chondroma	Chondrosarcoma
4. Osseous tissue	Osteoma	Osteosarcoma
5. Mucous tissue	Myxoma	Myxosarcoma
6. Fat tissue	Lipoma	Liposarcoma
7. Smooth muscle tissue	Leiomyoma	Leiomyosarcoma
8. Striated muscle tissue	Rhabdomyoma	Rhabdomyosarcoma
9. Endothelial tissue	Hemangioma	Hemangiosarcoma
	Lymphangiona	Lymphangiosarcoma
		Reticulosarcoma
	Endothelioma	Endotheliosarcoma
10. Lymphoid tissue		Lymphosarcoma
		Multiple lymphocytic myeloma
		Multiple plasmocytic myeloma
11. Myeloid tissue		Multiple myeloblastic myeloma
		Multiple erythroblastic myeloma
C. *Covering, Secreting and Innervating Tissues*		
	Epitheliomas	
1. Surface epithelial tissue and 2. Glandular epithelial tissue	Papilloma and Adenoma	Carcinoma

The papillomas may be subdivided according to the cell type present into

a. Spinous cell papilloma with cornifications
b. Spinous cell papilloma without cornifications
c. Round cell papilloma
d. Columnar cell papilloma

The adenomas may be subdivided according to their general structure into

a. Simple adenoma
b. Cystadenoma
c. Papillary adenoma
d. Papillary cystadenoma

The carcinomas may be subdivided as follows:

I. Primary solid carcinomas

a. Spindle cell carcinoma
b. Round cell carcinoma
c. Spinous cell carcinoma without cornifications
d. Spinous cell carcinoma with cornifications

II. Glandular carcinomas

a. Malignant adenoma
b. Gelatinous adenocarcinoma
c. Papillary adenocarcinoma and papillary cystadenocarcinoma
d. Simple adenocarcinoma and simple cystadenocarcinoma
e. Solid adenocarcinoma

III. Adenospinous cell carcinoma

While these expressions will sufficiently characterize the blastomas of ectodermal and endodermal origin in a general way, it may become necessary to modify or supplement these terms for the purpose of defining and classifying the different varieties of neoplasms revealing evidence of organic structural resemblances.

ORGAN	BENIGN TYPE	MALIGNANT
1. Skin	Spinous cell papilloma without cornifications	A. Epidermoid carcinoma
a. Epidermis	Spinous cell papilloma with cornifications	a. Basal cell carcinoma Spindle cell carcinoma Round cell carcinoma Adenocystic carcinoma b. Baso-spinous cell carcinoma c. Spinous cell carcinoma without cornifications Spinous cell carcinoma with cornifications
b. Hair follicle		B. Trichocellular carcinoma
c. Sweat glands	Syringoma Syringocystadenoma	C. Syringocarcinoma
d. Sebaceous glands		D. Sebaceocarcinoma
2. Mucous membranes covered with squamous epithelium		
		Lymphoid round cell carcinoma
	Round cell papilloma	Papillomatous round cell carcinoma
	Spinous cell papilloma without cornifications	Spindle cell carcinoma
	Spinous cell papilloma with cornifications	Round cell carcinoma
		Spinous cell carcinoma without cornifications
		Spinous cell carcinoma with cornifications
3. Mucous membranes and ducts covered with cuboidal or columnar epithelium		
	Columnar cell papilloma	Papillomatous columnar cell carcinoma
	Polypous adenoma	Glandular carcinomas of different type
		Argentaffinocarcinoma
4. Pigment-forming tissue		
	Melanoma	Melanocarcinoma of pigmented and nonpigmented, spindle cell and round cell variety
5. Nervous tissue	Glioma	Gliocarcinoma
	Neuroma	Neurocarcinoma
6. Chorda tissue	Chordoma	Chordocarcinoma
7. Serosa epithelium		Coelocarcinoma
8. Cortical adrenal tissue	Hypernephroma	Hypernephrocarcinoma
9. Liver tissue		Hepatocarcinoma
		Cholangiosarcinoma
10. Testis		Testiculocarcinoma
11. Ovary		Folliculocarcinoma
		Granulosa cell carcinoma
		Oophorocarcinoma
12. Tooth band	Adamantinoma	Adamantinocarcinoma
13. Chorionic epithelium	Chorioma	Choriocarcinoma
etc.	etc.	etc.

COMPOSITE BLASTOMAS

1. Composite blastomas of mesodermal tissue elements

Benign Type	*Malignant Type*
a. Leiomyofibroma	Myofibrosarcoma
b. Osteochondroma	Osteochondrosarcoma
c. Fibrolipoma	
d. Myxochondroma	Myxochandrosarcoma
etc.	etc.

2. Composite blastomas of mesodermal and endodermal or ectodermal tissue elements

a. Adenofibroma	Adenosarcoma
b. Adenomyoma	Adenomyosarcoma
c.	Carcinosarcoma
d.	Myxochondrofibrocarcinoma
e. Neurinofibroma	
etc.	etc.

In the composition of these terms, it should be a rule that the last part of the word refers to the predominating or most significant (malignant) element of the tissues of which the neoplasm consists. Following this principle an adenofibroma is a tumor which is composed mainly of fibrous tissue with an admixture of glandular tissue.

BLASTOMATOID GROWTHS

A. Benign blastomatoid growths
1. Teratomas (fetus in fetu, embryoma, simple teratoma, dermoid cyst).
2. Tissue misplacements (pseudo-erosion of cervix (congenital), islands of stomach mucosa in esophagus, cholesteatoma, endometrioma, nevus, etc.)
3. Tissue metaplasias.
4. Hyperplasias (adenomatoid formations of mucous membranes and glandular organs such as intestine, endometrium, liver, prostate, thyroid, etc.), (adenofibromatoid formations such as found in the breast), (fibromatoid formations such as found in the skin and fibromatoid polyps of mucous membranes [nose, sinuses]), (excessive regenerative hyperplasias such as amputationeuroma, keloid, etc.), (inflammatory granulomas of sarcomatoid structure such as xanthogranuloma [xanthosarcoma] and giant cell granuloma [giant cell sarcoma], condylomata acuminata, etc.).
5. Retention cysts of various kinds (follicular cyst of ovary, colloid cyst of thyroid, ranula, arteriosclerotic cyst of kidney, etc.).
6. Angiectases, such as lymph cysts, teleangiectasis, etc.
7. Diffuse glandular cystic degenerations (kidney, liver, ovary, breast, etc.).

B. Malignant blastomatoid growths
1. Chloroma, leucosarcoma, myeloid, and lymphoid leucemia.
2. Lymphogranulomatosis.
3. Mycosis fungoides.
4. Molluscum contagiosum.
5. Multiple hemorrhagic sarcoma (Kaposi).

generalized metastasis on the other side (round cell sarcomas, anaplastic carcinomas). This marked difference in clinical behavior of malignant tumors has led to the development of the biologic classification, as the clinical need was felt that these biologic differences should find some definite expression in the report of the pathologist.

c. Biologic classification. Biologic morphologic correlations have been made the basis of this classification. The degree of structural and cellular differentiation and anaplasia is used for the grouping of the tumors into several groups of potential histologic malignancy, which are supposed to express the varying degrees of the infiltrative, proliferative and metastatic tendencies of the different tumors. Broders has attempted to solve this problem by grouping the malignant neoplasms of the various organs into four grades of malignancy according to the relative number of undifferentiated cells present. Others have followed his lead by using the same or a somewhat modified method (Ewing, Martzloff, Greenough, etc.).

The method of the "histological malignancy-index" by me, contains twenty factors pertaining to the characteristics of the parenchyma and stroma of a tumor which are evaluated on a numerical basis. The sum of the values thus obtained represents the malignancy-index which is apt to be high in tumors of high malignancy and low in those of less malignant qualities. The following is a list of the factors and their values:

Histologic Malignogram	Name: Date:	Origin: Diagnosis:	
I. Special cellular and structural characteristics of carcinoma:			Valued 5-20
1. Special cell type of carcinoma		valued 1-4	
2. Nucleo-cytoplasmic coefficient		valued 1-4	
3. Number of "pencil-cells"		valued 1-4	
4. Infiltrative growth of carcinoma		valued 1-4	
5. General type of carcinoma (parenchyma-stroma relation)		valued 1-4	
II. Characteristics of cytoplasm:			Valued 5-20
6. Irregularity in size of cells		valued 1-4	
7. Irregularity in shape of cells		valued 1-4	
8. Distinctness in outline of cells		valued 1-4	
9. Chromatism of cytoplasm		valued 1-4	
10. Functional activity of cells		valued 1-4	
III. Characteristics of nuclei:			Valued 6-24
11. Irregularity in size of nuclei		valued 1-4	
12. Irregularity in shape of nuclei		valued 1-4	
13. Chromatism of nuclei		valued 1-4	
14. Hyperchromatism of nuclei		valued 1-4	
15. Number of mitoses and prophases		valued 1-4	
16. Irregularity of mitoses		valued 1-4	
IV. Characteristics of stroma:			Valued 4-16
17. Character of stroma		valued 1-4	
18. Vascularity of stroma		valued 1-4	
19. Type of cellular infiltration		valued 1-4	
20. Amount of cellular infiltration		valued 1-4	
Histological malignancy-index		20-80	

While there still exists a lively controversy in regard to the actual value of the grading of tumors, a recent statement of Ewing on this subject seems to characterize the problem in an illuminating way. He says: "I have little sympathy with the pathologists who, because of lack of knowledge or intellectual laziness, discard the matter of grading tumors as of no value and unsound. As industry increases and experience enlarges, most of these men will gradually see that they are finding an opportunity to serve the clinicians, which they should embrace under all circumstances."

The practical value of this method is that the clinician gets some information in regard to the probable prognosis and may intelligently choose the type of treatment. The grade of histologic malignancy, however, is certainly not the only factor on which the type of treatment should be based and the prognosis estimated. The extent of the disease as best expressed by definite clinical grouping (as done by Schmitz) the age of the individual, heredity, the general condition of the patient, the location of the tumor, its extrinsic or intrinsic growth qualities deserve as much consideration as the histologic grading. In a recent study the combined effect of extent and malignancy-grade upon the prognosis has been demonstrated. It is obvious that the histologic grade is useless for the determination of the probable outcome or the best type of treatment in a case of generalized carcinomatosis, and to judge the value of the method of grading without due consideration of such undeniable facts is incorrect. None of the different methods of grading are entirely free from the influence of the

personal equation and the results of every one of them represents a group grading and not an absolute grading of the individual case.

In regard to the estimation of the prospective radiosensitivity of malignant tumors on the basis of their histologic structure the "law of Bergonié" which claims that the radiosensitivity increases with the degree of immaturity of the tumor has found many supporters. This law has been somewhat modified lately by Regaud who substituted for the "degree of immaturity" the factor "intensity of metabolism." Others rely upon the frequency of mitoses (karyokynetic index) in their estimates of radiosensitivity. Schmitz and myself have been unable to demonstrate the validity of the law of Bergonié in tumors of the

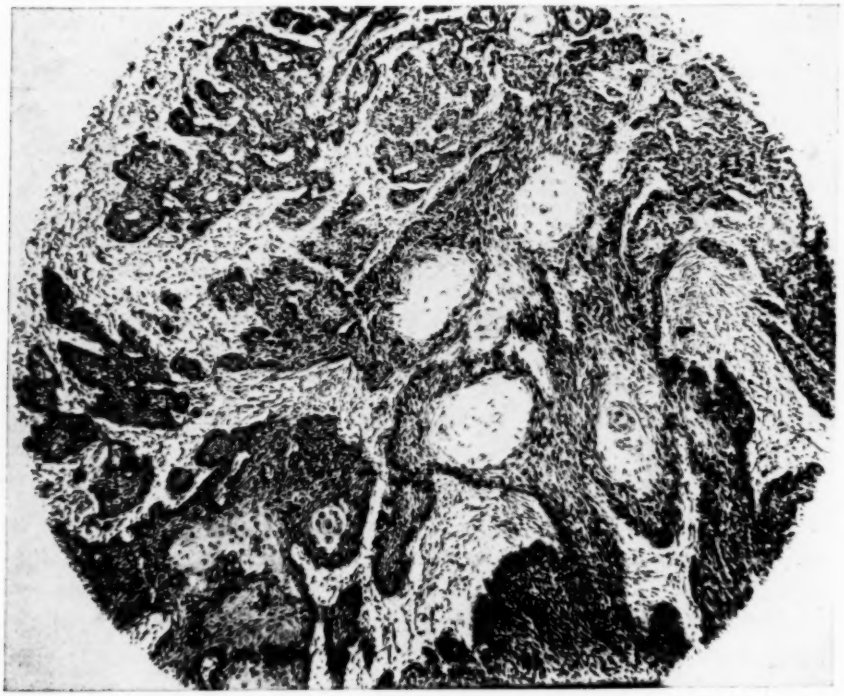

Fig. 13.—Basospinous cell carcinoma of the skin.

cervix and the breast. Others have made similar observations. While the primary effect of roentgen and radium rays upon highly immature and anaplastic tumors, which are frequently also very cellular, may be grossly more marked than that seen in mature tumors which contain often a larger amount of an inert substance (cornification, mucus, fibrous tissue, etc.) the permanent curative results obtained in undifferentiated blastomas are according to my own observations and those of others, more or less worse than those seen in the mature types. It must, however, be stated that highly malignant tumors with their tendency to spread and form metastases will evidently be more efficiently attacked and eradicated by the method of irradiation on account of the physical nature of the rays penetrating the whole area of the tissue involved than by surgical means.

The degree of malignancy is, however, in my opinion, the deciding factor in regard to prognosis. In estimating the degree of radiosensitivity not only the parenchyma of the tumor, but also the condition of the stroma (vascularity, fibrosis, bone formation, type and amount of leucocytic and lymphocytic infiltration, edema) must find proper consideration. The inclusion of these features in the "histological malignancy-index" is one of the points by which my method may represent an improvement.

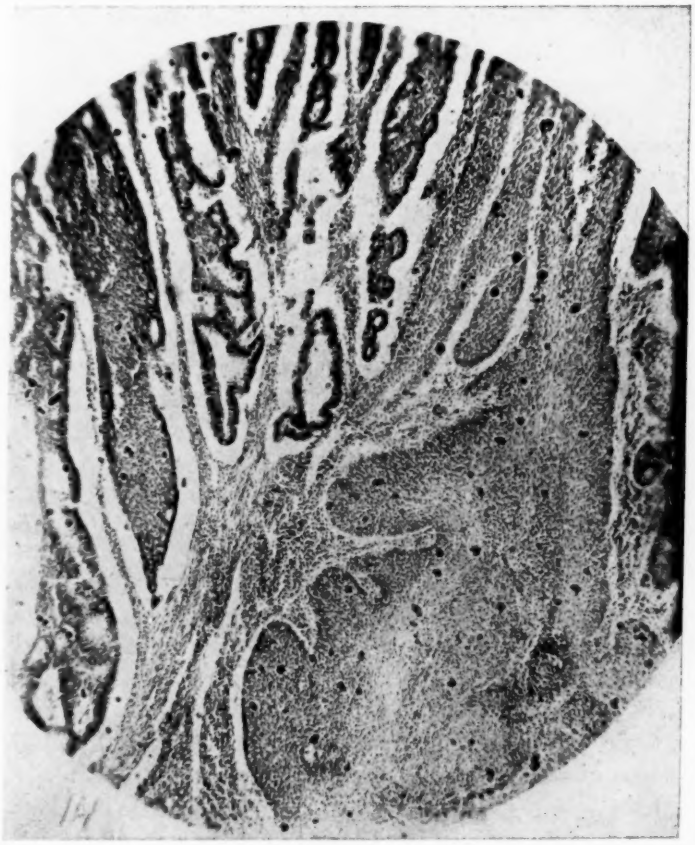

Fig. 14.—Adenospinous cell carcinoma of the skin.

When the information obtained by an adequate classification, malignancy determination and estimation of radiosensitivity of a given tumor is combined a complete pathologic diagnosis should read for instance as follows:

Ulcerated papillary adenocarcinoma of the uterine cervix. Malignancy Grade III. Radiosensitivity Grade II.

The diagnosis of cancer should not be done according to fixed conceptions in regard to the evaluation of the histologic picture without proper clinical check as to the correctness of such a procedure. The neglect of this consideration is a cause of much confusion in the differentiation of the so-called "precancerous" and carcinomatoid lesions from actual malignancy and in the sometimes difficult

distinction between benign and malignant tumors of the same histologic type. Our present diagnostic methods are insufficient and do not allow in all instances of these precancerous and pseudocancerous conditions a definite answer. Many points of this subject are still highly controversial (leucoplakias, chronic cystic mastitis, chronic gastric ulcer, etc.). Intimate familiarity of the pathologist with the characteristics of these lesions and close cooperation with the clinician will insure in these cases a high degree of reliability of the histopathologic diagnosis.

Attention should be called to the occurrence of slender, spindle-shaped or oval cells with elongated, spindle-shaped, hyperchromatic nuclei and a small amount of cytoplasm, either pointed on both ends or thickened on that end which points to the periphery of the carcinomatous cell nest. These cells invade the carcinomatous cell pegs from the periphery, being radially arranged, forming sometimes nets and splitting up the solid masses of carcinoma cells. In other places, they crowd the center of the strands where necrosis of carcinoma cells is present. These cells were first described by Lahm who named them "pencil-cells" and thought them to be pathologic carcinoma cells which attack the normal cancer cells and destroy them. He sees in their presence an attempt of the cancer toward a spontaneous cure. Babes and myself have made similar observations in cancers of the uterine cervix. But both believe that they are not atypical cancer cells. They have been found in cancers of various organs and in many typical and atypical epithelial proliferations of hyperplastic nature. Cervical cancers with abundant pencil-cells appear to have a better prognosis than cancers without these cells. They represent apparently a defensive reaction of the epithelium against the development and growth of cancer cells.

CONCLUSIONS

1. A scientifically sound and clinically practical classification of tumors prevents misunderstandings between pathologist and clinician and insures an efficient histologic diagnostic service and the possibility of comparative studies in tumor pathology and therapy.

2. Histologic grading of tumors stimulates an intimate histologic investigation of neoplasm and gives some definite information to the clinician.

3. The histological grade of malignancy is not the only factor on which the prognosis of a tumor depends. Others are equally important.

4. The occurrence of "pencil-cells" in hyperplastic and malignant growth is described and their probable prognostic significance discussed.

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MODIFICATION OF WILSON'S FRESH FROZEN SECTION TECHNIC*

By Albert C. Broders, M.D., Rochester, Minn.

WITH the use of Bethe's methylene blue intra vitam stain as a base, Wilson, in 1905, conceived and developed a much needed method of rapidly preparing fresh tissues for microscopic examination.

WILSON'S TECHNIC

Equipment.—The following materials are necessary: a Spencer automatic microtome with a carbon dioxide attachment in which vulcanite is substituted for brass in the wall of the freezing chamber in order to insulate the freezing plate; a tank of carbon dioxide; dextrin solution prepared in a special way (dry dextrin stirred in boiling water until the mixture is about the consistency of commercial maple syrup, to which is added phenol, 0.5 per cent, if the weather is warm); Brun's glucose solution, prepared in a special way (thoroughly hot distilled water 140 c.c., glucose 40 gm., and glycerin 10 c.c., are mixed; to this is added camphorated spirits, 10 c.c., and the mixture is shaken and filtered; a crystal of thymol is added if the weather is warm); 1 per cent sodium chloride solution; Unna's polychrome methylene blue; a clear glass dish such as a heavy, shallow, watch-glass in which to unroll sections; an eye-dropper and 3-ounce (90 c.c.) bottle for dextrin; a white, porcelain dish of a capacity of about 5 ounces (150 c.c.) for salt solution; an oval porcelain dish (undecorated match safe) or porcelain dish, 10 cm. long, 3 cm. wide and 2.5 cm. deep; a white porcelain match tray for Brun's glucose solution; a small porcelain teacup from a set of doll's dishes or a small individual glass saltcellar for polychrome methylene blue; clean slides in a container; clean cover glasses in a container; a camel's-hair brush, as is used by artists, and a small glass rod drawn out and bent at a convenient angle, the bent portion of which should be less than 1.5 cm. long with the end rounded, one surgical scalpel and one small tissue forceps.

Technical Procedure.—The piece of fresh tissue should be about 2 mm., 10 mm., and 10 mm. in various diameters. Blocks of tissue should be so trimmed that the section, when cut on the microtome, will be rectangular or rounded rather than triangular. If the block is not more than 1 cm. in transverse diameter, the long edge is placed on the freezing plate and in such a position that the edge of the knife will be parallel with the edge of the tissue; if it is more than 1 cm. in transverse diameter, the shorter edge should be placed so that it will first come in contact with the knife. A drop or two of dextrin solution is placed on the freezing plate of the microtome, with an eye-dropper; then the tissue is placed on the dextrin. If a minute bit of tissue is to be frozen, a small amount of dextrin is put on the freezing plate, is partially frozen there, and then the tissue is placed on top of it, thereby keeping the tissue 1 mm. or more away from the metal freezing plate in order to avoid contact with the knife. The microtome is set to cut sections 5, 10, or 15 microns thick; the carbon dioxide is turned on in intermittent spurts, thus

*From the Section on Surgical Pathology, The Mayo Clinic.

allowing the tissue to freeze slowly and evenly throughout. If tissue should freeze more solidly than necessary for cutting, the upper layer is thawed by placing a finger on it. Sections are cut by rapidly repeated strokes, not permitting the surface of the tissue to thaw after each cut. Six or more sections are cut and the first sections are rejected, thus insuring sections of even and uniform thickness. Sections are removed from the knife with the tip of the finger and allowed to thaw thereon; the process of thawing may be hastened by breathing on them, thus rendering less likely the formation of air bubbles than if the sections are removed with the brush and placed immediately in the salt solution. As sections go into salt solution they are usually more or less rolled up, and should be straightened out by gentle manipulation, by moving them up and down with a camel's-hair brush, or with a bent glass lifter with a dull point. The straightening out is best accomplished in a clear glass dish (a heavy shallow watch-glass) over a black background. After the section is straightened out it is caught under the middle by the bent portion of the small glass rod lifter and is transferred to the stain. Direct up and down motion is made with the lifter, the section remaining thereon as a rule, the folded portion "flapping" in the stain; this procedure is continued for ten to twenty seconds. A small receptacle for stain will greatly aid recovery of the section if it is lost in the stain. The section is washed in several ounces of salt solution which is contained in a white porcelain dish, and washing is continued as long as the stain comes away freely. It is then transferred to Brun's glucose solution which is contained in an oval porcelain dish of such a size that a 3-inch slide will rest in it, in a slanting position, one end on its bottom and the other on its edge. A long, narrow porcelain dish is considered superior for the Brun's glucose solution. The section is moved about in the glucose for a few seconds in order to straighten it out and to obtain better differentiation. It is then mounted from the glucose medium in the following manner: The end of a slide is slipped under it; its edge is held to the slide by the lifter, and the whole preparation is raised out of the solution, the excess glucose medium is wiped from underneath the slide and around the section, the cover glass is dropped on, and the preparation is ready for microscopic examination. All steps of the process can be carried out in one minute from the time the tissue is placed on the freezing plate of the microtome until the slide is placed on the stage of the microscope.

MODIFIED TECHNIC

Equipment.—The following materials are necessary: a Spencer automatic microtome, with a carbon dioxide attachment; a tank of carbon dioxide; one surgical scalpel; one small tissue forceps; one glass rod lifter, 15 cm. in length and 0.6 cm. in width, which is rounded at both ends; two or three Pyrex custard dishes, each 9 cm. across the top, 5 cm. across the bottom, and 3.5 cm. in depth; one China bird bath, 11 cm. in length, 7 cm. in width and 2.5 cm. in depth; a rectangular, glass slide holder, 9 cm. long, 7 cm. wide, and 4 cm. deep; two saltcellars of large size, one for the stain and the other for the cover slips; clean slides and cover slips; Terry's neutral polychrome methylene blue, and cool tap water.

Technical Procedure.—The block of tissue is prepared in the same manner as in the Wilson technic. It is dipped in tap water and placed on the microtome stage; the tissue carries over with it to the microtome stage enough water sufficiently to imbed it subsequently in ice. If the piece of tissue to be cut is very small, a drop of water can be utilized on the microtome stage in the same manner as that in which the dextrin is employed in the Wilson technic. The tissue is placed on the stage to the best advantage according to the judgment of the technician. In cutting a piece of tissue with skin on one side of it, it is always best to place the cutaneous surface nearest to the knife. The microtome is set to cut sections 15 microns thick. The tissue is held firmly in contact with the stage during the process of freezing. The carbon dioxide is turned on in intermittent spurts, as in the Wilson technic, until about half of the tissue is frozen. The carbon dioxide is then shut off and the tissue is cut with the microtome immediately above the frost line. The knife is now wet with water and is kept wet while slices 15 microns thick are cut. It is very important to have the tissue properly frozen. If

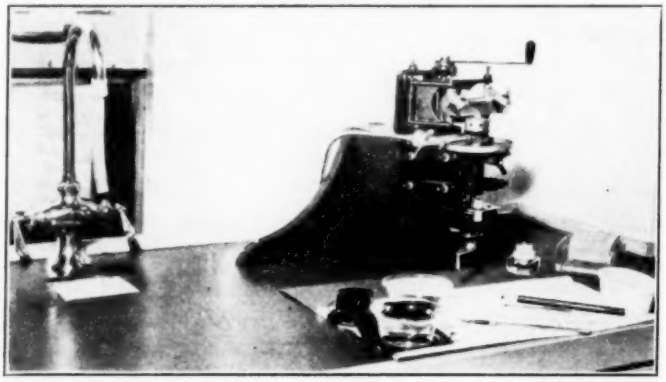

Fig. 1.—Equipment used in modified Wilson frozen section technic.

it is too hard, the sections will become fragmented; if it is too soft, they are likely to be torn. Experience only will teach one how to keep the tissue at exactly the right temperature. However, the water on the knife is a great aid in doing this. As soon as sections are cut, they are inspected, and imperfect sections are discarded. When a good section is obtained it is removed from the blade of the microtome with the tip of the index finger, which has been wet with water, and is transferred directly to one of the Pyrex dishes which is filled with water. This dish should be painted black on the bottom, or a piece of carbon paper should be placed under it. The section flattens out on the surface of the water and can be seen easily. It is picked up by inserting the end of the glass rod in the water beneath the section and then gently raising the rod, on which the section will lie smoothly. It is now transferred to the dish of Terry's neutral polychrome methylene blue; as the rod is dipped slowly into the stain the section floats off. This permits the entire undersurface of the section to come in contact with the stain. The section is again picked up on the rod and is immediately lowered to the stain, on which the section again floats. The cells of the section have such an avidity for the stain that complete penetration of it seems to take

place rapidly. As soon as the section appears to be satisfactorily stained, it is transferred on the rod to one of the dishes of tap water for washing purposes. When the rod is lowered into the water, the section floats on the surface. It is picked up on the rod again and again, by an up and down motion, until the technician feels that it is sufficiently washed of the excess stain. This procedure not only washes the excess stain from the section, but also

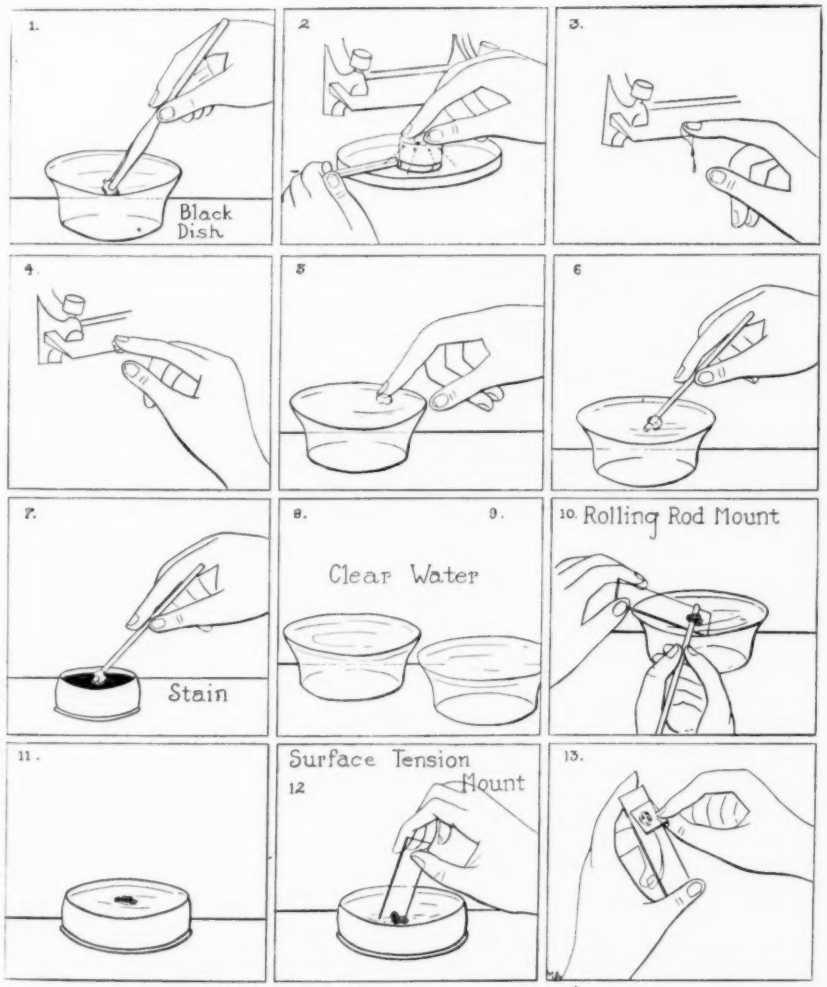

Fig. 2.—Various steps of the modified technic. *1* Dipping tissue in water. *2* Freezing tissue. *3* Wetting knife. *4* Removing section from knife. *5* Placing section on water. *6* Picking section up on lifter. *7* Placing section on surface of stain. *8* and *9* Wash waters. *10* Rolling rod or Ward method of mounting. *11* Section floating on surface of water. *12* Surface tension or Pulford method of mounting. *13* Placing cover glass over section.

cleans the stain from the rod. The section is removed from the tap water that is used for washing and is placed in the bird bath, which is also filled with tap water.

From this water the section is mounted in one of two ways, according to the choice of the technician. By the "rolling rod" method of mounting, the slide is immersed halfway in the water; the rod, with the section lying

on it, is brought to the edge of the water line on the upper surface of the slide; then, when the rod is rolled toward the slide, the section begins to float on the water, and its edge is immediately caught on the slide; the transfer is completed by continuing to roll the rod toward the slide, at the same time drawing the latter out of the water as fast as the section unrolls; this ingenious method of mounting was developed by a technician in this section of the clinic. The second method of mounting takes advantage of surface tension, which is greater in cool water than in warm water. The section, as it floats on the surface of the water, is dipped up, so to speak, by putting the slide in the water until it touches the bottom of the dish; then it is leaned toward the operator at an angle of about 45°. The slide is now brought in contact with the edge of the section, which adheres to the surface of the slide as the latter is withdrawn from the water. This method of mounting was devised in this section of the clinic by Dr. D. S. Pulford, who also introduced the method of surface staining with the aid of a large rod. Although both methods of mounting the section usually avoid wrinkling, with the rolling rod method, wrinkling is probably less likely to develop. The placing of the cover glass is the same after both methods of mounting. The cover glass is picked up, and one edge of it is put on the wet slide close to the section; it is then lowered gently until it comes in contact with the section. Should bubbles be caught beneath the cover glass, they can be forced out by applying slight pressure to the cover glass. Heavy pressure is always to be avoided, since it is likely to injure the section. After the excess water has been wiped from the slide, the section is ready for microscopic examination.

The chief advantage of the modified technic is that tap water is substituted for the dextrin, the physiologic salt solution, and the Brun's glucose solution. The handling of the section on the large glass rod permits it to be passed from water to stain and back to water without any violence, and furthermore prevents the formation of wrinkles. The section is in sight all of the time, and therefore is not likely to be lost in the stain. Finally, both the surface tension and rolling rod methods of mounting represent distinct improvement. All of the steps in the modified technic can be accomplished by a good technician with excellent results, in from forty-five to fifty seconds.

COMMENT

As a direct result of Wilson's original achievement, and its later modification, the properly trained surgical pathologist has become of inestimable value to the surgeon during the course of many operations. The fresh tissue method enables the pathologist to elucidate many vague pathologic processes while the patient is on the operating table, thereby, not infrequently, changing the previously planned operative procedure.

REFERENCE

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